Current Status and Dynamics of Anticoccidial Sensitivity and Resistance in Commercial Turkey Flocks

Dissertation

Presented in Partial Fulfillment of the Requirements for the Degree Doctor of Philosophy in the Graduate School of The Ohio State University

By

Hafiz Muhammad Abdullah, M.S.

Graduate Program in Animal Sciences

The Ohio State University

2024

Dissertation Committee:

Dr. Alejandro Relling, Advisor

Dr. Lisa Bielke

Dr. Ali Nazmi

Dr. Thaddeus Ezeji

Abstract

The current dissertation addresses the growing concern of resistance to anticoccidial medications in *Eimeria* affecting commercial turkey production in the US. This research aims to assess the efficacy of various anticoccidial medications by blending contemporary research with a historical data review to understand resistance dynamics. Fecal samples from turkey farms (n = 24) in the Midwest region of the US were collected between 2019 and 2023 for anticoccidial susceptibility testing of *Eimeria*. The research tested samples against six commonly used anticoccidial medications: Amprol (Amprolium), Avatec (Lasalocid), Coban (Monensin), Coyden (Clopidol), Stenorol (Halofuginone Hydrobromide) and Zoamix (Zoalene). To complete each test, anticoccidials were administered to independent groups of turkeys that were inoculated with test samples and were monitored for oocyst shedding, which was then compared back to a non-treated group to calculate the relative oocyst output.

A completely randomized block design and the SAS 9.4 Proc GLIMMIX procedure were employed for the quantitative evaluation of medication efficacy based on oocyst count reduction, with significance established at $P \leq 0.05$. Our findings indicate that Coyden outperformed the other medications with sensitivity against *Eimeria* detected in 54.17% (13 out of 24 farms) except Stenorol which showed intermediate effect staying between Coyden and other medications with 33.33% (5 out of 15 farms) sensitivity. Contrarily, Zoamix 4.7% (1 out of 21 farms), Amprol 9.5% (2 out of 21 farms), Avatec 9.5% (2 out of 21 farms) and Coban 9.5% (2 out of 21 farms) shown significantly lesser sensitivities. Coban indicated reduced sensitivity in 42.86% (9 out of 21 farms) , while Amprol and Stenorol each exhibited reduced sensitivity in 38.10% (8 out of 21) and 46.67% (7 out of 15) of farms, respectively. Moreover, Zoamix, Avatec, Amprol, and Coban exhibited the highest resistance, with 76.19% (16 out of 21 farms), 71.43% (15 out of 21 farms), 52.38% (11 out of 21 farms), and 47.62% (10 out of 21 farms) showing resistance, respectively. These results were then compared with temporal trends in sensitivity, reduced sensitivity, and resistance to the anticoccidial medications from the data published between1980-2024. We assessed both the impact of various medications and the influence of time on sensitivity across all tested anticoccidials. The data shows a significant decrease ($P < 0.01$) in sensitivity to anticoccidial medications by 1.23% per year since 1980-2024. Conversely, no differences in reduced sensitivity ($P = 0.32$) and resistance ($P = 0.13$) were observed. In addition to this, our results highlight the importance of continued surveillance and research efforts to monitor medication efficacy and resistance trends accurately. The observed decrease in medication sensitivity over time emphasizes the critical need for careful anticoccidial use, and the exploration of alternative coccidiosis control methods to sustain commercial turkey health and productivity.

Dedication

Dedicated to the pillars of wisdom and strength in my life-my beloved mother Professor Dr. Rubina Baqir, and my revered father Professor Rao Baqir Ali Khan. Their scholarly legacy, steadfast support, guidance, and intellectual influence have been the bedrock of my professional career and academic pursuits. This dissertation stands as a testament to the profound impact of your love and intellect in my life.

In honor of the indomitable Rajpoot tribes, particularly those of 53-2L, Okara, Pakistan, I pay special tribute to my forefather Prithvi Raj Chauhan, who fearlessly stood up for truth, freedom and nation leaving behind a legacy of unwavering principles and valor.

This dissertation is also dedicated to the resilient and indomitable spirits who fought and stood against racism, injustice, discrimination, and inequality in the United States of America. May their strength inspire ongoing efforts towards equality, understanding, a more just and inclusive society for all.

Lastly, this dissertation is a tribute to the citizens of diverse cultures, religions, colors, and ethnicities who collectively weave the intricate fabric of the American nation. May this diversity serve as a beacon, fostering understanding, harmony, and the enduring spirit of unity that makes this country “The United States of America”.

Acknowledgments

I extend my sincerest gratitude to my advising committee Dr. Alejandro Relling, Dr. Lisa Bielke, Dr. Ali Nazmi, and Dr. Thaddeus Ezeji for their invaluable guidance and support throughout the dissertation process. Dr. Relling's expertise in statistics provided crucial insights, while Dr. Bielke's meticulous attention to detail and encouragement for innovative approaches shaped the research. Dr. Nazmi and Dr. Ezeji's unwavering support was instrumental in the success of this endeavor.

I am profoundly grateful to my family—Sara, Ayesha, Abdul Rehman, Suhaib, Salman, Anum, Mujtaba, Abdul Muizz, Eshaal, Shifa, Hussain, Muhammad, Rubina, Muaviah, Ayat, Zubair, Zarrar—for their unwavering support and encouragement. A special acknowledgment goes to worlds best sister Hafiza Hafsa Nayyab for her exceptional support and understanding throughout my life.

I extend special thanks to my friends Nimra Khalid and Shuja Majeed, whose presence was a constant source of encouragement and joy and appreciate the collaborative spirit and dedication of my lab mates, Tony, Philip and Demi, whose contributions have greatly enriched our research endeavors.

A heartfelt gratitude to my mentors and fatherly figures, Dr. Anthony Parker, and Dr. Michael Lilburn, for their invaluable guidance, unwavering support, and mentorship.

I am truly privileged to have had such a remarkable support system. As I move forward, I carry with me the lessons learned, the bonds forged, and the enduring gratitude for the community. Thank you for being part of my journey.

Vita

2010…………B.S. Poultry Science, University of Agriculture, Punjab, Pakistan

2013………M.S. Animal Nutrition, University of Agriculture, Punjab, Pakistan

2012-2014…Assistant Production Manager, Pak Poultry Farms, Punjab, Pakistan

2014-2018…………………… Production Manager, Kreider Farms, Pennsylvania

2018-2022……….Poultry Operations Manager, The Ohio State University

2022-2023……………………...…Sr. Production Manager, Trillium Farms, Ohio

2023 to present………........................Business Unit Leader, Trillium Farms, Ohio

Publications

1. Zhou Y, Li Y, Zhang L, Wu Z, Huang Y, Yan H, Zhong J, Wang LJ, **Abdullah HM,** Wang HH. Antibiotic Administration Routes and Oral Exposure to Antibiotic Resistant Bacteria as Key Drivers for Gut Microbiota Disruption and Resistome in Poultry. Front Microbiol. 2020 Jul 7; 11:1319. Doi: 10.3389/fmicb.2020.01319. PMID: 32733394; PMCID: PMC7358366.
2. **Abdullah H,** Bielke L, Helmy Y. Effect of Arginine Supplementation on Growth Performance, and Immunity of Broilers: A Review. Journal of Global Innovations in Agricultural and Social Sciences. 2019 Dec 30; Vol 7: 141-144 Doi: 10.22194/JGIASS/7.879

3. Hussain A, Tian-tian WU, Fan LJ, Wang YL, Khalid MF, Jiang N, Gao L, Li K, Gao YL, Liu CJ, Cui H, Pan Q, Zhang Y, Aslam A, Muti-Ur-Rehman K, Arshad MI, **Abdullah HM,** Wang X, Qi X. The circulation of unique reassortment strains of infectious bursal disease virus in Pakistan. Journal of Integrative Agriculture, Volume 19, Issue 7, 2020; Pages 1867-1875, SSN 2095-3119.
4. Audrey F. Duff, W.N. Briggs, J.C. Bielke, K.E. McGovern, M. Trombetta, **H. Abdullah,** L.R. Bielke, K.M. Chasser. PCR identification and prevalence of *Eimeria* species in commercial turkey flocks of the Midwestern US, Poultry Science, Volume 101, Issue 9, 2022, 101995, ISSN 0032-579.
5. Fawad Ahmad, Asad Sultan, Sarzamin Khan, Majid Ali, Ihsan Ali, **Hafiz Abdullah,** Gamaleldin M. Suliman, Ayman A. Swelum,. Effect of citrus peeling (Citrus sinensis) on production performance, humoral immunity, nutrients, and energy utilization of broiler quails, Poultry Science, Volume 103, Issue 1,2024,103207, ISSN 0032-5791, https://doi.org/10.1016/j.psj.2023.103207.

Fields of Study

Major field: Animal Sciences

Table of Contents

List of Tables

List of Figures

Chapter 1: Introduction

Coccidiosis is the leading parasitic disease initiated by intracellular protozoa of the genus *Eimeria*. It mainly affects the intestine of susceptible hosts, causing economic losses related to high morbidity, malabsorption, poor feed conversion and higher mortality in poultry (Dalloul et al., 2005) ultimately compromising food reliability (Kadykalo et al., 2018). Amongst the most expensive and widespread diseases of poultry globally, coccidiosis results in annual losses that have been projected to exceed USD 3 billion (Stevens, 1998; Williams, 1999; Dalloul and Lillehoj, 2006; Kadykalo et al., 2018). Growth of the poultry industry is projected to double by the year 2050 (Alexandratos and Bruinsma, 2012) and it is extremely important to control this disease as poultry is primarily fulfilling the rising food demands by an increasing world population (Kart and Bilgili, 2008). The control of coccidiosis using anticoccidial medication is necessary to accomplish continuous and cost-effective poultry production and they have been utilized for preventive measures since the late 1940s (Rathinam and Chapman, 2009). The extensive occurrence of antimicrobial resistance has provoked concerns in modern poultry production regarding the safety of anticoccidial medications and their probable impact on human and animal health as well as the environment (Zidar and Žižek, 2012). Despite improvements in genetic methods and biotechnical and immunological techniques, control of this disease primarily relies on prophylactic chemotherapy with anticoccidials, especially for turkey production where vaccines are not widely available (McDougald and Reid, 1994). However, the development of resistance in *Eimeria* species against anticoccidial medication is an ongoing risk to the continuous success of prophylactic chemotherapy (Williams, 2006). Within a few years, *Eimeria* can develop resistance to almost every compound that has been introduced (Chapman, 1997). Since ionophores comprise almost 80% of anticoccidial use for past 30 years, being resistant to them has turned out to be an increasing challenge all over the world (Chapman, 1986; McDougald et al., 1986; Rotibi et al., 1989; Vertommen and Peek, 1993 and McDougald et al., 1996). Therefore, the development of newer and active anticoccidial medication which can replace the older medication has become enormously difficult.

Commercial turkey production plays a great role in fulfilling food demands producing more than 2.67 billion kg of meat/year in the US (USDA 2023). Some *Eimeria* species have been observed to cause clinical coccidiosis in commercial turkeys; however, evidence on the extent of pathogenicity is lacking (Imai and Berta, 2019). In the U.S. Rathinam and Chapman (2009) conducted a survey on *Eimeria* isolates from commercial turkey flocks, 31/36 *Eimeria* samples were resistant against amprolium, 23/36 were resistant to monensin, 10/36 and 6/36 were resistant to diclazuril and clopidol respectively. Surprisingly, 2/36 samples were resistant to all the medications and 26/36 samples were observed to be multi medication resistant. Thus, it is crucial to investigate the efficiency of anticoccidial medications since accurate and rapid identification of loss of sensitivity may possibly direct the medication usage and assist veterinarians to make better choices in management of resistant protozoa and prolong the life of several anticoccidial medications. Ultimately, this investigation will allow more turkeys to reach processing with less material input than if flocks were treated blindly at the risk of applying a resistant anticoccidials.

Chapter 2: Literature Review

2.1. Introduction to coccidiosis

Coccidiosis is a parasitic disease of the intestinal tract of poultry that is caused by protozoan parasites of the genus *Eimeria*. The disease is attributed to protozoa, specifically belonging to the phylum Apicomplexa, subclass Coccidia and the suborder *Eimeriina,* family *Eimeridae* or the *Eimeria* (Shivaramaiah et al., 2014). The genus *Eimeria*, which belongs to the subclass coccidia, is particularly significant for poultry species. According to Duszynski et al. (2018) about 1200 different species of *Eimeria* have been identified, and each has a distinct effect on various intestinal tract segments.

Coccidiosis is of worldwide occurrence and every year costs the poultry industry millions of dollars to control. The disease spreads from one animal to another by contact with infected feces or ingestion of oocysts. Bloody diarrhea is the primary symptom of coccidiosis in turkeys. Turkeys infected with coccidia show poor growth, loss of appetite and weight, suffer severe symptoms such as immunosuppression, and bloody diarrhea which ultimately results in death of the turkeys.

This protozoan parasite inflicts significant harm to the turkeys by initiating the partial or complete deterioration of epithelial cells that line the intestinal tract, a condition referred to as intestinal coccidiosis. Mostly, coccidiosis occurs during the humid seasons characterized by abundant rainfall (Lilić et al., 2009) and is prevalent amongst poultry and various other animals.

2.2. *Eimeria* of Poultry Industry

Eimeria infections have a significant impact on the poultry industry, resulting in economic losses of approximately USD 1.36 billion to 4.72 billion kilograms annually in worldwide broiler production (Blake et al., 2020). In the UK, the cost of coccidiosis to chicken production was estimated to be $123.05 million per annum, 95.1% of which was derived from broiler production (Blake et al., 2020). In the Romanian broiler industry, coccidiosis is responsible for economic losses ranging from 95.6% to 98.1% (Györke et al., 2016). Pawestri et al. (2020) reported that the estimated direct loss due to coccidiosis in Indonesian broiler chicken was $229.26 million. A recent study by Gilbert et al. (2020) explored the economic effects of *Eimeria* infections and their control measures within intensive broiler farming systems across the Europe. Without any intervention to control *Eimeria*, the study found that the median production losses per square meter of broiler space ranged from $2.73 to $3.25.

Between the late 1950s-late 1980s, the prevalence of *Eimeria* spp in poultry was studied by several scientists. Hein (1974), however, proposed a link between high pathogenicity and *Eimeria* prevalence in poultry, particularly broiler chicken. Braunius (1988) emphasized the significant influence of *Eimeria* prevalence on economic performance, particularly in broiler farms using Zoamix. Braunius (1988) also reported that *E. maxima* infections were correlated negatively with the production broiler farms using Zoamix. Table 2.1 outlines the scientists focus on determination of the *Eimeria* prevalence in poultry amongst different regions of the World.

Table 2.1 Country-wise Prevalence of *Eimeria* spp. in Poultry

Country	Type of Animal	Prevalence reported	Reference
Australia	Broilers	98% Commercial and 81% in backyard flocks.	Godwin and Morgan, (2015)
Brazil	Broilers	96% Commercial	Moraes et al., (2015)
Canada	Broilers	2.5% Commercial	Ogedengbe et al., (2011)
China	Broilers	*Eimeria* is 90%, 88%, 72%, 68%, 60%, 26%, and 8% for *E. tenella, E. praecox, E. acervulina, E. maxima, E. mitis, E. necatrix,* and *E. brunetti,* respectively.	Sun, et al., (2009)
China	Domestic Chicken	87.75%	Huang et al., (2017)
China	Broilers	30.7% Commercial	Lan et al., (2017)
China	Broilers	97.17%.	Geng et al., (2021)
Ethiopia	Domestic Chicken	56%.	Luu et al., (2013)
India	Broiler	57.57%	Rao et al., (2012)
Iran	Broilers	55.96%	Nematollahi et al., (2009)
Iran	Broilers	31.5%	Hamidinejat et al., (2010)
Iran	Domestic Chicken	64%.	Hadipour et al., (2011)

Continued

Table 2.1: Continued

Iran	Broilers	31.50%	Hamidinejat et al., (2010)
Iran	Domestic Chicken	64%.	Hadipour et al., (2011)
Japan	Breeding Chicken	51.50%	Matsubayashi et al., (2020)
Jordan	Broilers	50%.	Al-Natour et al., (2002)
Korea	Broilers	78.7%.	Lee et al., (2010)
Myanmar	Domestic Chicken	33.6%.	Bawm et al., (2021)
Netherlands	Broilers	50.3%.	Braunius, (1988)
Nigeria	Poultry farms not specified	36.7% among adult birds 52.9% among younger birds.	Muazu et al., (2008)
Nigeria	Domestic Chicken	42.7%.	Adang and Isah, (2016)
Nigeria	Poultry	18.9% Layers	Ola-Fadunsin et al., (2019)
		11.11% Broilers	
		50% Turkey	
Nigeria	Domestic Chicken	158 (39.5%)	Okwuonu et al., (2021)
Nigeria	Poultry	54.4% Broilers 62.3% Layers	Asaaga et al., (2022)
Norway	Broilers	91%	Györke et al., (2013)
Pakistan	Broiler Layer Breeder	71.86%	Khan et al., (2006)
Saudia Arabia	Breeding Chicken	63.8%.	Al-Taee et al., (2018)
South Africa	Broilers	59.50%	Fatoba et al., (2020)
U.S.A. (Alabama)	Backyard Chicken Flocks	64.1%	Carrisosa et al., (2021)
U.S.A.	Wild Turkeys	66%	Ruff et al., (1988)

As shown in Table 2.1 that the prevalence of *Eimeria* in poultry has been extensively studied, with findings indicating variations in incidences, pathogenicity, and species distribution across the globe and time periods, though this was not evaluated in detail to determine if the apparent differences are real. These studies have provided valuable insights into the impact of *Eimeria* infections on poultry health and productivity. Continued research and surveillance are crucial for understanding the evolving prevalence patterns, identifying emerging strains or species and their life cycles, and developing effective control measures to minimize the economic losses associated with *Eimeria* infections in poultry and 50 years after Hein's suggestion, the need for consistent surveillance continues (Hein, 1974).

2.3 Overview of Poultry Industry: Focus on Chicken and Turkeys

The poultry industry is a significant contributor to the US economy, with chicken and turkeys being the primary sources of meat and eggs.

Chickens are raised for both meat and egg production with broilers being the most common type of chicken raised in the US. According to USDA (2023), the total sales values from chicken industry in 2022 was approximately $77 billion with broilers being the major contributor having $50.4 billion in sales and total live weight produced was approximately 26.7 billion kg. On the other hand, layer industry produced approximately 110 billion eggs valuing approximately $19.4 billion whereas sales from rest of the chicken industry such as breeder industry, back yard chicken, specialty chicken sold in local and niche markets was $74.7 million.

The US is home to a variety of turkey breeds, which have been developed over time for meat production (Harper and Kime, 2021). The turkey production is concentrated in states like Minnesota, North Carolina, Arkansas, Indiana, Missouri, Virginia, Iowa, Pennsylvania, and Ohio. The top three states in turkey production for 2021 were Minnesota, North Carolina and Arkansas, with a total production of 216.5 million birds (USDA Economics, Statistics, and Market Information System, 2023). According to National Turkey Federation (2023) turkey consumption within the US, as measured by disappearance has a projected decline to 2.2 billion kg in 2022 while per capita consumption has also experienced a gradual decrease, with an estimated value of 6.62 kg in 2022. In terms of trade, turkey meat exports from the US totaled 248.57 million kg in 2021, with a projection of 189.15 million kg in 2022 (National Turkey Federation, 2023). Presumably, this decline in consumption is likely related to turkey meat cost compared to chicken, and development of strategies to improve efficiency of turkey meat production may help reduce the price of meat, thus providing opportunities for increased consumer purchasing.

The poultry industry faces various challenges, including disease outbreaks, environmental concerns, and animal welfare issues. One of the most significant challenges is the outbreak of coccidiosis, which causes significant economic impact on the farmers. The industry has implemented various measures to prevent and control the spread of coccidiosis, including biosecurity measures, vaccination, and anticoccidial medication use.

Schering-Plough Animal Health Corporation (2003) reported that the turkey industry faces unique challenges in managing coccidiosis compared to the chicken industry due to least choices in vaccination, medication, and their longer production cycle resulting in their potential exposure to the disease for a longer period than chicken. Nevertheless, vaccination and medication are extensive methods to combat coccidiosis, there is only one approved coccidiosis vaccine named Immucox-T, available for turkeys (Milbradt et al., 2014) which covers only two *Eimeria* species, *E. meleagrimitis* and *E. adenoides*. Turkeys are infected by 7 known *Eimeria* species; among those, 4 are considered highly pathogenic i.e. *Eimeria meleagrimitis, Eimeria adenoides, Eimeria gallopavonis*, and *Eimeria dispersa*, whereas the remaining species, *Eimeria innocua, Eimeria meleagridis*, and *Eimeria subrotunda*, cause comparatively less losses due to their low pathogenicity (Milbradt et al., 2014).

Most of the coccidia control products in the US are ionophores (55%) and chemical anticoccidials (33%) (Clark and Pyle, 2009) while coccidia vaccination is limited to 7% only. The low incidence of vaccination might be due to the restricted availability of the only USDA-approved commercial turkey coccidiosis live vaccine.

On the other hand, coccidiosis is also common and economically significant disease in both layers and broilers. The disease causes subclinical infections as well as mortalities in persistently infected flocks, leading to reduced weight gains, drop in production, and poor feed conversion ratios (Gerhold, 2016; Kadykalo et al., 2018). The infection has been reported from all types of commercial chicken (broiler, layer, breeder) ranging 7%–90% (Zaheer et al., 2022). Amongst all *Eimeria* species of chicken, *E.*

maxima, E. tenella, E. acervulina, and *E. necatrix* have reportedly the highest clinical and sub-clinical disease burden, making them economically significant compared to the other species.

The susceptibility to coccidiosis can vary between layers and broilers. The longer growth cycle of layers increases their potential exposure to the disease for a longer period (Iqbal and Begum, 2010). However, both broilers and layers are affected by coccidiosis, and the disease has a significant impact on production in both cases. The chicken industry due to its demand and significantly larger economic contribution, has advantage over turkey industry. Thus, having more options in using live virulent, attenuated, and recombinant vaccines with more *Eimeria* strains in a single vaccine than turkeys (Liu et al., 2023). Different methods have been used to administer coccidiosis vaccines to poultry, including feed, drinking water, coarse spray, solid gel pucks, or in ovo vaccination (Shivaramaiah et al., 2014). Several vaccines are on the market to combat coccidiosis in broilers and layers. One such vaccine, Coccivac-B52, is a live vaccine that includes a blend of five different *Eimeria* species (*Eimeria acervulina, Eimeria maxima, Eimeria maxima MFP, Eimeria mivati* and *Eimeria tenella*) known to cause coccidiosis in broilers (Jenkins et al., 2023). Another live vaccine called Immucox, contains a mix of three *Eimeria* species (*Eimeria acervulina, Eimeria maxima,* and *Eimeria tenella*) responsible for coccidiosis in broilers (Arczewska-Włosek et al., 2022).

Conversely, these chicken vaccines are not directly applicable in the case of turkeys due to the presence of additional *Eimeria* species affecting turkeys. This difference in susceptibility highlights a significant challenge in turkey vaccine development, as a result, Immucox-T attempts to bridge this gap, yet it still does not provide comprehensive protection against all the *Eimeria* species affecting turkeys (Imai, 2018; Trujillo-Peralta et al., 2023). The specificity of host-parasite interaction among *Eimeria* species and their poultry hosts underlines the complexity of creating cross-protective vaccines. Reflecting on both historical and contemporary research, it is clear that cross-protection between *Eimeria* species across different poultry species is limited, underscoring the importance of developing species-specific vaccines to effectively manage coccidiosis across poultry industry (Moore and Brown, 1951, 1952; Hawkins, 1952; Moore et al., 1954; Vrba and Pakandl, 2014; Imai and Barta, 2019).

2.4 Life Cycle and Pathogenesis of Coccidia in host health

The lifecycle of *Eimeria* within a particular host is 5-7 days, depending on species, with the entire cycle occurring within a singular animal. It involves several stages, including sporulation, ingestion, invasion, schizogony, gametogony, and oocyst excretion. During the sporulation phase, *Eimeria* withstands harsh environmental conditions, such as dryness, and temperature variability making it more critical stage for survival and proliferation of the parasite. Initially, unsporulated oocysts with thick shells, containing a single nucleus in a large protoplasm pool, are excreted in feces. Under suitable environmental conditions such as high humidity (60% to 80%) and optimal temperature (25°C to 30°C), sporulation occurs as the nucleus undergoes division, which results in the formation of polar bodies around each nucleus (Venkateswara et al., 2015; Répérant et al., 2021). These polar bodies separate from the central core, giving rise to sporoblasts. Simultaneously, the protoplasm develops a sporocyst wall, enclosing two sporozoites within it. This entire process, under optimal conditions, typically takes 2 to 4 hours, but it may be prolonged if the conditions are unfavorable (Cuomo et al., 2009; Kheysin et al., 2013)

Oocysts initially contain one or two sporoblasts which undergo a series of divisions that are specific to the species of coccidia, ultimately transforming into sporocysts. The exact number of divisions and resulting sporocysts is determined by the species of coccidia involved. For instance, each sporoblast typically divides to form two sporocysts, with each sporocyst containing four sporozoites. Therefore, the total number of sporozoites produced per oocyst can vary, but often, for many *Eimeria* species, this results in eight sporozoites per oocyst after the division process is complete (Dubey et al., 2019). Sporulation involves the development and maturation of oocysts, with sporoblasts differentiating into sporocysts (Al-Badri and Barta, 2012).

During invasion, once mature oocysts are ingested by a new host, they reach the gastrointestinal tract where sporocysts are released, and the location of release varies with species as can be noted by the location of infection. During excystation, sporozoites break through the plasma membrane of sporocysts, and these motile cells migrate along the epithelium to find cells to invade and begin intracellular replication (Augustine, 2001).

During the schizogony stage, sporozoites that have invaded epithelial cells undergo asexual reproduction which involves rapid multiplication through multiple divisions, leading to the formation of structures called schizonts. Sporozoites within schizonts divide widely, generating many daughter cells called merozoites (Attias et al., 2020). Often, the number of generations of schizogony is associated with pathogenicity, with more generations causing more damage to the gut. In case of *Eimeria meleagridis*, two generations of schizonts were identified, with the first generation developing mainly near the base of the villi and the second generation found at the sides of the villi but not in the crypts of Lieberkuhn (Chapman et al., 2004). *Eimeria meleagrimitis* exhibits three generations of schizonts, with the first and second generations observed along the sides of the villi and the third generation occurring concurrently with gametogony (Chapman, 2008). *Eimeria dispersa* shows four generations of schizonts, located along the sides and tips of the villi but not in the crypts of Lieberkuhn. *Eimeria adenoides* exhibits two asexual generations of schizonts, with the first generation observed at 48 hours along the sides of the villi and the second generation present at 84 hours (Clarkson, 1956, 1958), Limited information is available regarding the early stages of *Eimeria gallopavonis*, *Eimeria innocua,* and *Eimeria subrotunda* (Chapman, 2008).

2.5 Host Specificity, Factors, and Control Strategies of *Eimeria*

Eimeria species are recognized for their high host specificity which can be attributed to evolutionary adaptation and co-evolution with their respective host species. This specificity is influenced by a combination of factors. Firstly, the immune system of the host plays a critical role, as each bird species has a unique immune response to pathogens (Xiao et al., 2002). Secondly, the intestinal environment and the cellular architecture of the gastrointestinal tract vary significantly among different bird species. For instance, thickness of the intestinal wall, composition and density of villi, and presence of specific types of mucosal cells can influence the ability of sporozoites to attach, invade, and replicate within the intestinal cells. Thirdly, the molecular and biochemical interactions between the parasite and the host are finely tuned. Specific receptors in intestinal cells of the host may be required for the invasion by *Eimeria* sporozoites, and these receptors may vary or be absent in non-susceptible hosts.

Additionally, genetic factors and the evolutionary history of the host-parasite relationship contribute to the specificity. Over time, *Eimeria* species have evolved alongside their hosts, leading to a precise match between the parasite lifecycle and the biological environment in the. host. This co-evolutionary process ensures that *Eimeria* parasites are highly adapted to their specific hosts but may lack the necessary adaptations to successfully infect and reproduce in other species (Miglani et al., 2002).

Oocysts shed in excreta into litter become infectious material for subsequent animals, as hundreds of thousands of oocysts can be contained with a single dropping (Fatoba and Adeleke, 2018). This becomes important for control of coccidiosis because on fresh litter, oocyst load will be low, but as flocks age, can become much higher, leading to high morbidity and mortality if control measures are not successfully implemented. Immunity to infection takes about two weeks to develop, which represents more than one lifecycle of *Eimeria*, meaning farm managers must take special care to limit replication of oocyst and sporulation within litter during the gap vaccination and full immune protection. Anticoccidials can be provided to flocks to prevent from diseases but must be fed for the duration of the growing period, which becomes costly for turkey production with up to 20-week cycle.

Coccidiosis spread in turkeys depend upon factors such as the infective dose, environmental conditions, and host factors. Young turkeys are frequently exposed to contaminated litter, building structures, and equipment (Gadde et al., 2020). Sites may become contaminated because of previously infected flocks, as oocysts are difficult to eliminate because they are not susceptible to standard disinfectants. Notably, wet areas near waterers also serve as sources of infection. Oocysts have ability to withstand wide ranges of humidity and temperature, combined with minimal nutritional needs, they can remain viable in litter for several months. This enables the potential of farms to be repeatedly contaminated across years even if no new isolates or strains are introduced. Additionally, the litter environment often presents challenges to complete eradication, ensuring the persistence of oocysts and the continued risk of infection (Reyna et al., 1983; Williams, 1995). Furthermore, factors like high stock density, poor ventilation, improper handling of the litter, and inadequate lighting schedules can facilitate the rapid spread of *Eimeria* in farm environment. However, the oocysts can be eradicated or at least greatly reduced through extreme litter dryness, freezing, and high environmental temperatures between the flocks which impede coccidia growth by creating unfavorable environment for the parasite disrupting their lifecycle. Coccidiosis can be controlled by adapting different strategies like batch population management between grow-outs, improved ventilation, increased biosecurity measures, and keeping birds on wire floors to prevent contact with droppings. Anticoccidial medications are often used prophylactically, but their continuous use can lead to the emergence of medication resistance. Rotation and shuttle programs involving different anticoccidials are implemented to reduce resistance development.

Clinical signs of coccidiosis in poultry, includes severe bloody diarrhea, decreased feed and water consumption, weight loss, decreased growth rate, decreased egg production, high mortality, and potential development of necrotic enteritis caused by Clostridium perfringens, which can occur as a secondary infection in chicken (Kadykalo et al., 2018). The lesions resulting from coccidiosis are typically localized along the intestinal tract and possess distinct locations and appearances that are helpful in diagnosis (López-Osorio et al., 2020). Some of the characteristics of *Eimeria* species that infect turkey has been shown in Table 2.2.

Table 2.2: Characteristics of *Eimeria* Species Infecting Turkeys

Eimeria	Location	Pathogeny	Lesions
Large Intestine			
E. meleagridis	Caecum (mainly)	Low	Ulcerations, Content: white caseous plugs/fibrinous necrotic content
E. gallopavonis	Caecum (mainly)	Higher	Petechiae/thickening of distal ileum and rectum, ulcerations, Content: clotted blood, caseous plugs, yellow exudate over the ulcers
Small Intestine			
E. meleagrimitis	Small gut, mostly lower part (jejunum)	Medium	Thickening of jejunum, few petechiae, Content: watery, white mucus strands
E. dispersa	Small gut, mostly upper part (duodenum)	Low	Content: white mucus in duodenum, strong blood perfusion
E. innocua	Small gut, mostly duodenum	Low	Content: watery, white mucus like *E. dispersa*
E. subrotunda	Small gut	No	

Adapted from Vrba and Pakandl (2014). Some studies describe E. adenoides as a separate species, although Vrba and Pakadl (2014) classify it as E. meleagridis.

In diagnosing coccidiosis, laboratory techniques serve as a valuable tool, yet comprehensive diagnosis requires the integration of various factors such as presence and intensity of bloody diarrhea, and its impact on the overall productivity of the affected animals. The specific location of the lesions caused by coccidia can provide insights into the involved *Eimeria* species. For instance, Rampin et al. (2006) reported an outbreak of *Eimeria adenoides* involving high mortality in in 30-day-old commercial turkeys. They observed that *Eimeria adenoides* had caused severe typhlitis and large numbers of oocysts were found in ceca.

Within the laboratory setting, two primary types of analyses can be conducted to expand our understanding of *Eimeria* lifecycle and its impact on the host. Oocyst counting is the method involves quantifying the number of oocysts per gram of feces, providing an estimation of the severity of the infestation. However, it is worth noting that oocyst counts may not always directly correlate with the clinical condition. The species of *Eimeria* present, can be determined by carefully examining the oocysts under a microscope. Techniques like sugar or salt flotation can be employed for oocyst isolation, and tools like the McMaster chamber aid in quantification (Bauer et al., 2023). Histological examination of mucosal scrapings or intestinal samples is another approach. The samples are fixed using 10% formaldehyde, embedded in paraffin wax, sliced into thin sections, and stained with hematoxylin and eosin (H&E) for observation under a microscope (Mesa-Pineda et al., 2021). This technique provides insight into the tissue-level changes caused by coccidia infection.

2.6 Anticoccidial Medication and Resistance

Anticoccidial resistance is defined as a shift in susceptibility to an anticoccidial, occurs when a strain of *Eimeria* survives and/or multiplies despite being exposed to medication doses equal to or higher than the standard recommendations by World Health Organization (Chapman, 1984; Chapman 1993; Anthony et al., 2005; Abbas et al., 2009; Chapman and Jeffers, 2014). This resistance, classified into types such as acquired, cross, and multiple, evolves through heritable decreases in medication sensitivity over time.

Acquired resistance is directly related to the medication concentration, where a lower dose can lead to resistance development, as seen with medications like sulfaquinoxaline and polyether ionophores (Chapman, 1984; Chapman 1993). Cross-resistance involves resistance among different compounds sharing a similar action mode, often reported among ionophores anticoccidials like maduramicin, monensin, and salinomycin. Multiple resistance refers to resistance against multiple medications with different action modes, emerging from sequential exposure to these compounds. Factors responsible for this resistance development include genetic factors of the parasite, operational factors related to medication use, and biological factors related to the parasite life cycle and behavior (Henken et al., 1994; Williams, 2002; Christaki et al., 2004; Anthony et al., 2005; Kitandu and Juranova, 2006; Naidoo et al., 2008; Abbas et al., 2009)

The concept of refugia is significant, referring to parasite populations that escape medication treatment effects, thereby slowing resistance selection. Many indices including Global Index (GI), Anticoccidial Sensitivity Test (AST), Optimum Anticoccidial Activity (OAA), and Anticoccidial Index (ACI) are used to access the effectiveness of anticoccidial medications. These indices use factors such as weight gain, feed conversion ratio, lesion score, oocyst output, and survival rate to assess medication effectiveness (Usman et al., 2011; Chapman and Jeffers, 2014; Quiroz-Castaneda and Dantan-Gonzalez, 2015; Muthamilselvan et al., 2016). This evolving resistance landscape necessitates a continuous and dynamic approach to monitoring and managing anticoccidial medication use in poultry farming, ensuring the effectiveness of these medications in controlling coccidiosis (Sundar et al., 2017).

2.6.1 Trends and Patterns in Anticoccidial Resistance

To combat anticoccidial medication resistance, the poultry industry has adopted several approaches, including extending the use of existing medications and innovative programs like the rotation and bio-shuttle programs. These involve alternating the use of compounds with different modes of action; for instance, starting with a synthetic medication like nicarbazin followed by an ionophore. The rotation program consists of using anticoccidial vaccines or medications through succeeding flocks, showing improvements in feed conversion ratio and body weight gain. Contrarily, a bio-shuttle program involves periodically changing the class of anticoccidial medication during turkey production cycle (Quiroz-Castaneda and Dantan-Gonzalez, 2015; Muthamilselvan et al., 2016; Sundar et al., 2017). Restoring sensitivity is another approach, achieved by introducing medication-sensitive coccidia strains, typically through vaccines.

Recombinant vectored vaccines, which do not contain live parasites, are emerging as a safe and effective method of controlling coccidiosis. These vaccines use vectors to deliver target epitopes from key sporozoite and merozoite proteins (Blake et al., 2017). Edible vaccines, like those developed using antigens from *Eimeria* proteins expressed in plants like tobacco, have also shown promise in inducing strong immune responses and reducing disease severity in chicken. These innovative solutions are reshaping the approach to managing anticoccidial medication resistance, emphasizing the need for varied and integrated strategies to tackle this evolving challenge (Sundar et al., 2017).

Case studies in the US, Europe, and Canada have documented increasing resistance to anticoccidial medications in turkey production, leading to outbreaks of coccidiosis and economic losses. Chapman (2001) observed resistance to monensin, while Mathis and Broussard (2006) noted diclazuril resistance in the Midwest, US. Peek and Landman (2006, 2010, 2011) found resistance to stenorol in Europe, with McDougald and Fitz-Coy (2008) reporting diminished efficacy of various anticoccidials across European turkey farms. In Canada, Agunos et al. (2019) highlighted resistance to Avatec and salinomycin, recommending judicious medication use and surveillance. These studies emphasize the need for integrated management, including medication rotation, non-chemical control, and regular efficacy monitoring to manage coccidiosis sustainably.

2.7 Future of Coccidia Management

The future of coccidiosis management in poultry focuses on diverse strategies to combat this parasitic disease. In turkey coccidiosis management, several medications are approved, including divalent ionophores like lasalocid (Avatec®) and monovalent ionophores such as monensin (Coban®). Synthetic anticoccidials, for instance, zoalene (Zoamix®) or SDM+OMP (RofenAid®), are often incorporated in rotation or shuttle programs with ionophores to manage the disease effectively throughout the year.

Effective control in commercial turkey production involves rotating these medications. A true rotation entails switching between different classes of products, utilizing diverse modes of action to manage *Eimeria* species effectively and prevent resistance, thereby ensuring the long-term efficacy of these management tools. Failure to rotate can lead to increased infection rates and greater subclinical losses. Anticoccidials are typically added to feed from day one and withdrawn when birds reach 9 to 12 weeks.

Vaccination is a promising avenue, as turkeys can develop a significant immune response to coccidia. Coccidia vaccines, like Immucox® T by Ceva, containing wild-type turkey *Eimeria* species, are approved in the USA. Various delivery systems, including spray cabinets, eye drops, and edible materials, are being explored, with repeated exposure essential for developing immunity (Clark, 2019).

Additionally, the use of phytonutrients as dietary supplements is gaining traction. They can be administered via feed or water, either in conjunction with traditional anticoccidial programs or independently. Phytonutrients, including plant extracts, prebiotics, and essential oils, have shown potential activity against coccidia, and are increasingly incorporated into management strategies.

2.8 Data Availability and Research Gaps

The importance of the current study is underscored by the significant gaps in the literature regarding the management of coccidiosis in turkeys, particularly in the context of anticoccidial medication sensitivity and resistance. These gaps have a profound impact on the study, as they limit the availability of foundational knowledge necessary for developing effective treatment strategies for turkeys. Unlike chicken, for which there is an abundance of research on *Eimeria* species and their management, turkeys have received comparatively less attention, leading to a scarcity of data on turkey specific *Eimeria* strains and the efficacy of anticoccidial medications in these birds.

One of the critical reasons we cannot directly apply chicken-centric studies to turkey farming is the distinct differences between the *Eimeria* species that infect chicken and those that affect turkeys. These species-specific differences mean that resistance patterns and the sensitivity of anticoccidial medications observed in chicken do not necessarily translate to turkeys. This discrepancy highlights the need for turkey-specific research to understand the features of *Eimeria* infections in turkeys and to develop treatments that are effective for this particular poultry species.

The attributed predominance of data on chicken is due to its larger and more globally widespread industry leading to a higher volume of research funding and studies. In contrast, turkeys are primarily raised for meat production, and the industry, while significant but smaller in scale. This disparity in economic importance and industry size contributes to the uneven distribution of research efforts, resulting in more data on chicken compared to turkeys.

2.9 Hypothesis and objectives.

This study is hypothesized with following objectives.

Hypothesis 1:

Eimeria from Central US farms will have resistance against more than 50% of commercial anticoccidial medications.

Objective 1:

Evaluate susceptibility and resistance of *Eimeria* samples from commercial turkey flocks against six anticoccidial medications i.e., Amprol, Avatec, Coban, Coyden, Stenorol, and Zoamix.

Hypothesis 2:

Throughout the last several decades, as per previously published findings and those currently reported here on turkey *Eimeria*, there has been a significant increase in the frequency of anticoccidial resistance.

Objective 2:

Measure changes in anticoccidial resistance in commercial turkey flocks across decades, based on current data from our laboratory and published reports from regions in the US and Canada.

Chapter 3: Current status and dynamics of anticoccidial sensitivity and resistance in commercial turkey flocks of US

3.1 Introduction

Coccidiosis is the leading parasitic disease initiated by intracellular protozoa of the genus *Eimeria*. It mainly affects the intestine of susceptible hosts, causing economic losses related to high morbidity, malabsorption, poor feed conversion and higher mortality in poultry (Dalloul et al., 2005). Bird welfare has always been a concern during coccidial infections, which can ultimately compromise the feed conversion ratio (Kadykalo et al., 2018). Amongst the most expensive and widespread diseases of poultry, coccidiosis results in annual losses that have been projected to exceed USD 3 billion globally (Stevens, 1998; Williams, 1999; Dalloul et al, 2007; Kadykalo et al., 2018).

Control of coccidiosis using anticoccidial medications is necessary to accomplish continuous and cost-effective poultry production and they have been utilized for preventive measures since the late 1940s (Rathinam and Chapman, 2009). The extensive occurrence of antimicrobial resistance has provoked concerns in modern poultry production regarding the safety of anticoccidial medications and their probable impact on human and animal health as well as the environment (Zidar and Žižek, 2012; Nilsson et al., 2012). Despite improvements in genetic methods and biotechnical and immunological techniques, control of coccidiosis primarily relies on the prophylactic chemotherapy with anticoccidials, especially for turkey production where vaccines are not widely available (Chapman 2008; Peek and Landman, 2011). However, the development of resistance to

Eimeria species against anticoccidial medications is an ongoing challenge to the continuous success of prophylactic chemotherapy (Williams, 2006). Within a few years, *Eimeria* can develop resistance to almost every compound that has been used on a farm (Chapman, 1997). Since ionophores are extensively used to combat the coccidiosis challenges in the turkey industry, *Eimeria* resistance against the ionophores poses a serious challenge and without a resistance tracking program the turkey industry is under a risk of significant financial losses.

In Iran, field isolates of *Eimeria* showed limited efficacy of common anticoccidials like salinomycin, amprolium+ethopabate, and diclazuril (Arabkhazaeli et al., 2013) Similarly, in Nigeria, *Eimeria tenella* populations exhibited resistance to medications like amprolium hydrochloride, amprolium hydrochloride + sulfaquinoxaline-sodium, and toltrazuril (Ojimelukwe et al., 2018). Furthermore, research in South Korea revealed severe multi-medication resistance of *Eimeria* to various anticoccidials like coyden, diclazuril, maduramycin, coban, salinomycin, and toltrazuril (Flores et al., 2022).

Although, there are some data available for the prevalence of turkey *Eimeria* resistance against anticoccidial medication, surveys in the US show that field turkey *Eimeria* had resistance against all the major anticoccidial medications tested (Mathis et al., 2021; Trujillo-Peralta et al., 2023). Thus, it is crucial to investigate the efficiency of anticoccidial medications since accurate and rapid identification of resistance may possibly direct the medication usage and assist veterinarians to make better choices in management of resistant protozoa and prolong the life of anticoccidial medications within production systems.

The hypothesis of the current investigation is that *Eimeria* from Central US turkey farms will show resistance to more than 50% of commercially available anticoccidial medications. The objective of this study is to evaluate the susceptibility and resistance of *Eimeria* species from these farms against six targeted anticoccidial medications: Amprol, Avatec, Coban, Coyden, Stenorol and Zoamix, aiming to inform effective disease management strategies in commercial turkey production.

3.2 Materials and Methods

Sample Acquisition

Turkey litter and/or excreta samples were requested from turkey farms in the Midwest region of the US. Veterinarians and farmers received detailed instructions on how to collect the fecal samples, along with all necessary packing and collection supplies for submitting to the Poultry Enteric Health Research Laboratory at Ohio State University. Fresh droppings were collected from the top of the litter to ensure they represented the current flock and shipped overnight on cooler packs. Upon receipt, samples were mixed with 2% potassium dichromate (Sigma-Aldrich, St. Louis, MO) at a w:v ratio of 1:2 and placed on orbital shakers in Erlenmyer flasks to promote sporulation of oocysts. A total of 24 samples containing sufficient levels of *Eimeria* were received from the Midwest region of the U.S.

Detection and sporulation of *Eimeria* Oocysts

Initially, oocysts were detected using McMaster Method to determine concentration within samples. To quantify the total oocysts present, 1ml aliquot from each sample was diluted tenfold in saturated NaCl. A baseline threshold of 60,000 total oocysts was established to determine the suitability of the samples for sporulation, which ensured an adequate volume of sporulated, infective oocysts for subsequent dosing and cycling in nonmedicated poults. Samples meeting this criterion were then covered with perforated aluminum foil, to allow for air exchange, and placed on a rotary platform shaker set at 100 rpm and maintained at room temperature for approximately one week to facilitate the sporulation process (Imai and Barta, 2019).

Turkey Housing and Management

Experimental methods were carried out at the poultry research facility of the Ohio Agricultural Research and Development Center. Aviagen turkey poults were reared in floor pens with fresh pine shavings (n = 4 poults/pen; 4 replicate pens per sample) with *ad libitum* access to water and feed. Ambient temperature and lighting schedules were maintained within age-appropriate ranges throughout all experiments. Preventative measures against cross-contamination included ammonia footbaths, insect control, and strict barn access protocols. All experimental protocols were approved by the Ohio State University under IACUC protocol number 2019A000000138.

Anticoccidial Medication and Oocyst Inoculation in Poults

For oocyst inoculation, sporulated samples from each farm were diluted in 0.9% saline to a concentration of 1000 sporulated oocysts/mL (Snyder, 2021). On day 7, the poults were provided a diet containing one of six anticoccidial medications for testing against each sporulated fecal sample, plus a set of non-medicated control pens for comparison of oocyst output (n = 4). Anticoccidials included: Amprolium (Amprol, Huvepharma, Peachtree City, GA) at 2.5 mL/L of water, Lasalocid (Avatec, Zoetis, Kalamazoo, MI) at 113 g/US ton of feed, Monensin (Coban, Elanco, Greenfield, IN) at 90 g/US ton, Clopidol (Coyden, Huvepharma, Peachtree City, GA) at 225 g/US ton, Halofuginone (Stenerol, Huvepharma, Peachtree City, GA) at 2.72 g/ton and Zoalene (Zoamiz, Zoetis, Kalamazoo, MI) at 170.3 g/US ton. On day 9, poults (n = 4 poults/pen in 4 pens) were inoculated with 1 mL of oocysts from a designated farm via oral gavage. From day 4 - 7 post-inoculation, excreta were collected twice daily into cumulative jars respective to inoculum from the designated farm and were preserved in 0.9% saline at 1:2 (w: v) for oocysts per gram counts.

Oocysts per gram counts

To calculate dilution factors, the volume of each fecal sample was first increased by adding an equal volume of 0.9% saline. After that, the samples were further diluted with saline for the McMaster counting method. To ensure that oocysts were distributed evenly, the fecal material in the samples was well shaken before counting. Based on the oocyst counts, the anticoccidial sensitivity of every sample was determined. The following formula was applied in this calculation (Snyder et al., 2021):

% Reduction of Oocyst Shedding =

$$1 - \frac{Number\ of\ oocysts\ shed\ by\ anticoccidial\ group}{Number\ of\ oocysts\ shed\ by\ Positive\ control\ group} \times 100\%$$

This computation allowed for sensitivity of each anticoccidial medication to be divided into three groups: 30% to 79% reduction in oocyst shedding suggests decreased sensitivity (RS), less than 30% reduction in oocyst shedding indicates resistance (R), and a reduction in oocyst shedding exceeding 79% signifies sensitivity (S).

Statistical Analysis

Data were analyzed using a completely randomized block design in SAS (9.4), employing Proc GLIMMIX to assess the resistance, reduce sensitivity, and sensitivity of medications against *Eimeria*. The model accounted for fixed effects of medications and random effects of farms, considering a binomial distribution. The age of the turkeys at sampling was added as a covariate in the analysis and reported in Appendix A, but not reported in this chapter. Differences were described at a P value ≤ 0.05. If there was a significant difference, means were separated using fisher protected test, using the option PDIFF (pairwise differences) of SAS.

3.3 Results

Frequency Distribution analysis

Fecal samples from 24 commercial turkey farms located in the Midwest US were analyzed to assess the sensitivity of *Eimeria* species to six commonly used anticoccidial medications. The age of the turkeys ranged from 6 to 77 days, with an average age of 29.37 ± 16.21 days. The sensitivity levels were determined by comparing the oocyst shedding between non-medicated and medicated treatment groups, providing insights into the efficacy of the medications. The samples, submitted by veterinarians from commercial turkey farms, represent a broad age spectrum, offering a comprehensive view of *Eimeria* sensitivity across different growth stages. Table 3.1 illustrates the frequency distribution of sensitivity levels to various medications in the Midwest region of US farms between 2019 to 2023, categorized as pan-sensitive, pan-resistant, or multi-medication resistant based on their response to the anticoccidial treatments.

Table 3.1: Anticoccidial sensitivity classification of commercial turkey flocks tested between 2019 and 2023 across the US.

Anti-coccidial Sensitivity Classification	% Flocks Tested
Pan-sensitive	7%
Sensitive/reduced sensitivity ≥ 4	34%
Multi-anticoccidial resistant	55%
Pan-resistant	14%

Note: Eimeria samples from a total of 24 flocks of varying age were tested for anticoccidial sensitivity to up to six anticoccidial compounds (see Materials and Methods) and classified as sensitive, reduced sensitivity, or resistant. For these classifications samples were classified as pan-sensitivity if all samples were sensitive or reduced sensitive to all medications; multi-anticoccidial resistant if samples were resistant to ≥ 3 anticoccidials and pan-resistant if samples were sensitive to ≤ 1 anticoccidial.

Sensitivity

There was a treatment difference for sensitivity ($P < 0.01$), Coyden was more sensitive ($P < 0.05$) against the *Eimeria* compared to the other medication except Stenorol with observed sensitivities in 54.17% (13 out of 24, Figure 3.1). Stenorol 33.33% (5 out of 15 farms) had an intermediate sensitivity and was in between Coyden and the other medications such as Zoamix 4.7% (1 out of 21 farms), Avatec 9.5% (2 out of 21 farms), and Coban 9.5% (2 out of 21farms) showed lesser sensitivities compared to Coyden. Amprol 9.5% (2 out of 21 farms) appeared to be similar to Stenorol due to close significance values between both medication indicating that it could be a type II error.

Reduced sensitivity

There were no differences ($P = 0.29$) on reduce sensitivity for the different medications. Coban had a 42.86% (9 out of 21 farms), while Amprol and Stenorol each showed reduced sensitivity in 38.10% (8 out of 21) and 46.67% (7 out of 15) of farms, respectively (Figure 3.2)

Resistance

There was a difference in resistance to different medications ($P < 0.01$). Specifically, Zoamix, Avatec, Amprol, and Coban exhibited the higher resistance, with 76.19% (16 out of 21 farms), 71.43% (15 out of 21 farms), 52.38% (11 out of 21 farms), and 47.62% (10 out of 21 farms) showing resistance, respectively. Coyden, and Stenorol appeared to be the least resistant medications as illustrated in Figure 3.3.

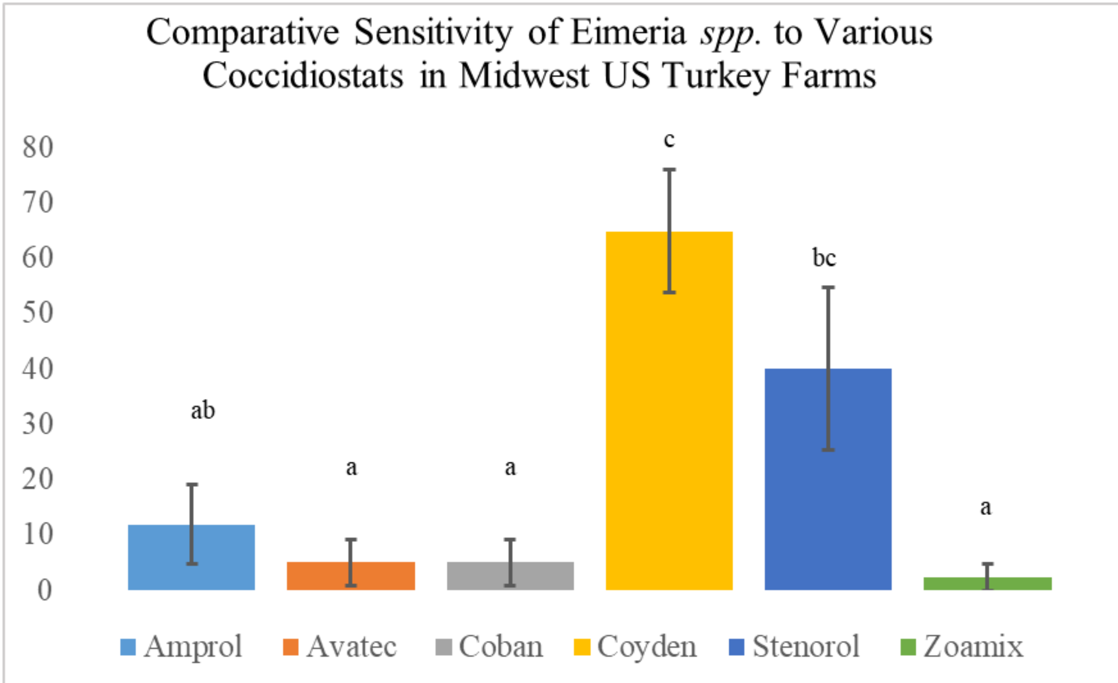

Figure 3.1: *Eimeria spp.* Sensitivity to Anticoccidial Medications in Midwestern US Turkey Farms. [abc] indicate differences in medication sensitivity ($P < 0.05$).

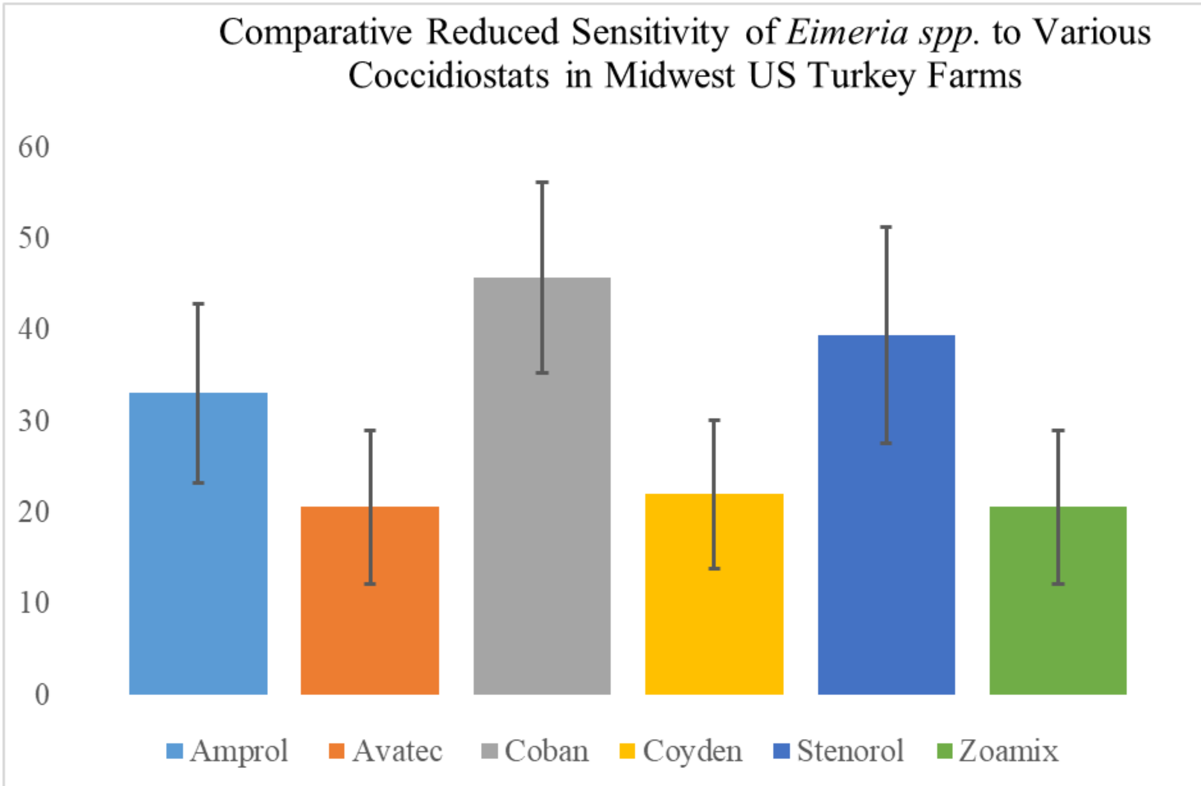

Figure 3.2: *Eimeria spp.* Reduced Sensitivity to Anticoccidial Medications in Midwestern US Turkey Farms (No differences in medication-reduced sensitivity, P = 0.29).

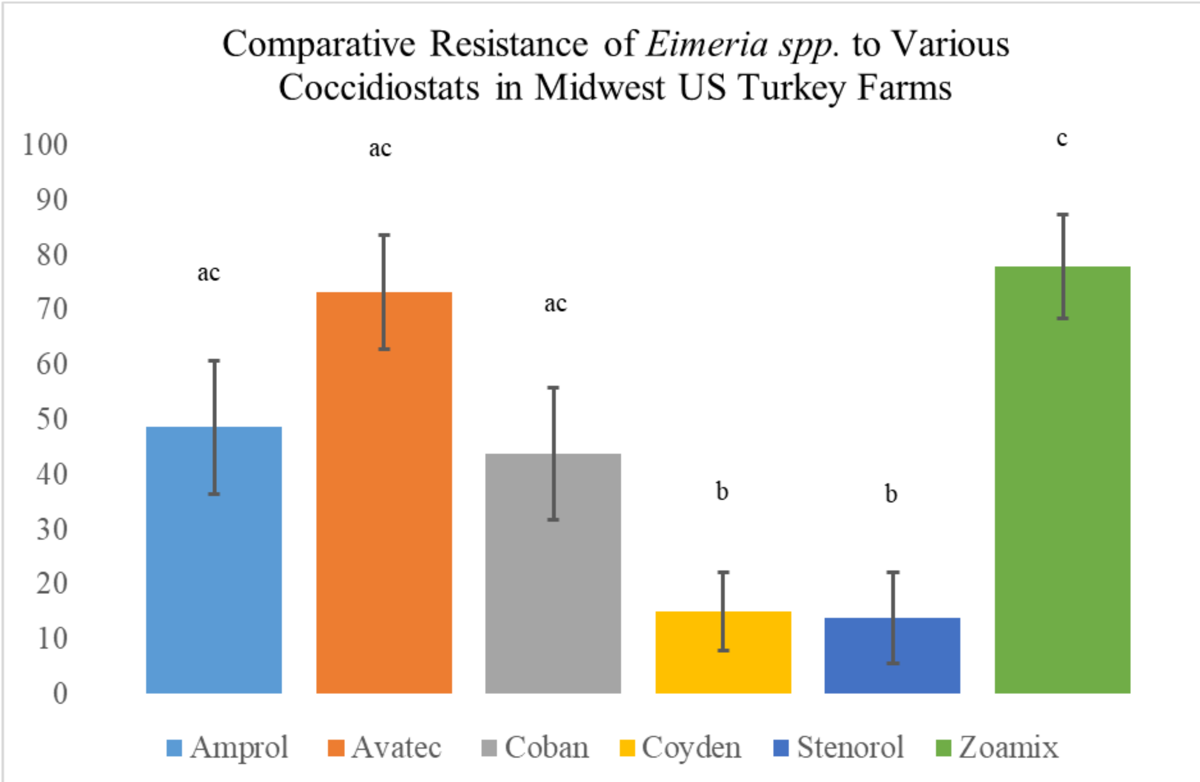

Figure 3.3: *Eimeria spp.* Resistance to Anticoccidial Medications in Midwestern US Turkey Farms. [abc] indicate differences in medication resistance ($P < 0.01$).

3.4 Discussion

The present study provides an insightful examination of the resistance profiles of *Eimeria spp.* across turkey farms in the Midwest region of the US, with a focus on the efficacy of six anticoccidial medications. Our findings reveal a significant variation in sensitivity and resistance among the tested anticoccidials, which has critical implications for the management strategies of coccidiosis in poultry farming. As summarized in Table 3.1, we observe a predominant trend towards multi-anticoccidial resistance, with 55% of flocks exhibiting this pattern. This underscores the urgent need for diversified management strategies.

Coyden demonstrated significant sensitivity in the farms, with sensitivity of 54.17% whereas, Coyden resistance level in our study was low with only 20.8% of the samples showing resistance in the commercial turkeys. Additionally, there was a 25% rate of reduced sensitivity. The comparatively higher sensitivity of *Eimeria spp.* in our study to Coyden might reflect less frequent use in the field, delayed development of resistance, or perhaps a combination of both factors. Our findings present a stark contrast to study of Huang et al. (2001), which reported a high level of complete resistance, with 80% of chicken isolates showing no response to Coyden. The diminished sensitivity of Coyden in the study conducted by Huang, can be attributed to *Eimeria spp*. developing resistance through continuous, prolonged exposure, possibly due to genetic mutations that allow the parasite to overcome metabolic blockage of the medication. This suggests that efficacy of Coyden could decrease over time, especially if used exclusively without rotation or in combination with other treatments. In the case of Stenorol which showed intermediate results staying between Coyden and other medications as 20% of the tested samples showed resistance and 46.7% of samples exhibited reduced sensitivity. However, 33.3% of the samples still retained sensitivity to Stenorol. The findings in our study are consistent with the conclusions drawn by EFSA (2022) who found Stenorol to be sensitive for both broilers and turkeys especially during their growth phases. Notably, Zhang et al. (2012) also reported that Stenorol played a significant role in reduction of *E.tenella* infections in commercial broilers. Additionally, studies conducted by Peek and Landman in 2006 and 2011 across European turkey and poultry farms showed an emerging resistance to Stenorol, likely due to improper medication cycling leading to persistent low-level exposure of the parasites to the medication, a condition known to foster resistance development. The mechanism of action for Stenorol inhibits the enzyme guanosine 5'-monophosphate (GMP) reductase which plays a pivotal role in DNA synthesis of coccidia meaning that Stenorol disrupts protein synthesis and development cycle of the parasite (Pines and Spector, 2015). Conversely, Coyden, targets, and inhibits mitochondrial energy production within the sporozoites (Bucek, 1969).

Our research also indicated a notable decrease in the sensitivity of Amprol and Coban, with more than half of the *Eimeria* population in these farms showing resistance to these medications. Specifically, Amprol had a 52.4% resistance rate and 38.1% reduced sensitivity, while only 9.5% of isolates remained sensitive. *Eimeria* samples demonstrated a similar pattern against Coban with 47.6% resistance, 42.9% reduced sensitivity, and 9.5% sensitivity. These findings are consistent with previous studies by McLoughlin (1970), Bi et al. (2018), and Arabkhazaeli et al. (2013), which reported varying degrees of reduced sensitivity to complete resistance to these medications. My research, in line with Peek and Landman (2003) and Rathinam and Chapman (2009), highlights significant resistance to medications like Amprol and Coban in the turkey industry. This echoes the patterns observed in the chicken industry, suggesting a widespread issue in poultry farming. The study conducted by Jeffers and Bentley in 1980, which examined the efficacy of Coban from 1976 to 1978, also identified a correlation between resistance and continuous medication usage. This correlation was particularly notable in Canadian turkey flocks as compared to those in the US. Our findings are further supported by Chapman (2008), who noted that 70% of US turkeys are exposed to Coban, leading to increased resistance and reduced sensitivity to this medication. The resistance patterns to anticoccidials like Amprol, introduced in the 1960s, and diclazuril, introduced in 2001, show a similar trend due to prolonged usage, as reported by Joyner and Norton (1969) and Peek and Landman (2003).

Amprol inhibits the thiamine metabolism and utilization in *Eimeria* because it lacks the hydroxyethyl group of thiamine inhibiting the conversion of thiamine into thiamine pyrophosphate and stops the phosphorylation in the *Eimeria* cells (Rogers, 1962; Ball et al., 1987; James, 1980). In contrast, resistance against Coban in *Eimeria* is attributed to alterations in its cell wall permeability, reducing cation transport (Jeffers, 1989; Chapman, 1997; Wang et al., 2006). Coban was the first ionophore introduced to the US poultry industry in July 1971 (Chapman et al., 2010). Even though the use of many anticoccidial compounds have been stopped due to *Eimeria* resistance against them, the use of Coban as coccidiosis control medication is continued in the industry.

Zoamix and Avatec exhibited the highest resistance levels among the *Eimeria* populations sampled, with over 75% and 71% resistance, respectively. The observed reduction in the efficacy of these medications in real-world applications aligns with prior research, which has documented a growing trend of resistance among coccidia to specific anticoccidial agents (Agunos et al., 2019; Bi et al., 2018). Historically, these same medications demonstrated substantial sensitivity against turkey coccidiosis, with Radu (2008) reporting notably low mortality rates. This underscores the initially favorable outcomes associated with the use of Avatec for the prevention of coccidiosis in turkey production. The notable resistance to Zoamix is of significant concern, especially since this medication has been a cornerstone in the treatment of coccidiosis for an extended period, as Trujillo-Peralta et al. (2023) point out. For instance, Braunius in 1988 already highlighted the considerable impact of *Eimeria* prevalence on the economic performance of farms, especially those employing Zoamix. This indicates that *Eimeria* was somewhat sensitive to Zoamix in the late 1980s. However, its sensitivity has drastically declined over time, as evidenced by our current study and numerous others (Xinyu et al., 2000; Bi et al., 2018). These studies show a trend towards complete resistance, with Xinyu et al. (2000) reporting a 100% resistance rate. Furthermore, by, Bi et al. (2018) also observed that Zoamix had lost all efficacy in treating *Eimeria*.

While this study has certain limitations, such as its reliance on data from commercial turkey farms and not fully exploring factors like farm management practices, environmental conditions, and specific *Eimeria* strains, its contributions to the field are significant. My findings play a crucial role in the current understanding of medication resistance in *Eimeria*. They underscore the necessity of ongoing surveillance and sensitivity testing, which are vital for the judicious use of anticoccidials. My research also sheds light on the pressing need to explore alternative management strategies, notably, vaccination plans, which can re-introduce anticoccidial sensitive strains onto farms.

Conclusion

Based on the findings of the current chapter, Coyden stands out as the more sensitive medication against *Eimeria* in the current context, while the use of Amprol, Avatec, Coban, Zoamix, and Stenorol might require careful consideration due to the high resistance levels observed. This information is crucial for veterinarians and poultry farm managers in making informed decisions about anticoccidial medication strategies to ensure effective and sustainable control of coccidiosis.

Chapter 4: Analyzing Anticoccidial Medication Efficacy and Temporal Patterns of Sensitivity and Resistance in Commercial Turkey Production: A Mixed Model Analysis Across Three Decades

4.1 Introduction

Coccidiosis is a widespread and economically significant disease affecting the turkey industry, causing considerable economic losses. The disease is caused by various species of the protozoan genus *Eimeria* and is responsible for high mortality and morbidity rates, as well as reduced production performance in turkeys. It is responsible for significant economic losses in the livestock industry (Noack et al.,2019). According to a report by Sandu (2023), coccidiosis is estimated to cause economic losses exceeding $3 billion globally. Recent recalculations, based on 2016 prices, indicate that the total cost of coccidiosis in the U.S. alone amounts to approximately $1.57 billion. Coccidiosis damages the intestinal mucosa of birds, leading to diarrhea, growth retardation, and decreased feed efficiency (Chapman, 2008). The prevalence of coccidiosis in turkeys is a major concern for producers, as it poses significant challenges to achieving optimal productivity in turkey production (Vrba and Pakandl, 2014). Shuttle programs that rotate anticoccidial medications and vaccines can limit resistance, but the availability of only a handful of medication options for turkeys affects the effectiveness of this strategy.

Anticoccidial medications play a crucial role in managing and preventing the impact of coccidiosis on turkey health and productivity. These medications are essential for controlling the disease and mitigating economic losses in the poultry industry (Kadykalo et al.,2018). They can be administered from the first day of the birds' life up to just before slaughter, depending on withdrawal periods, to protect against reinfection due to the omnipresence of the stadium oocystic disease. The most commonly used anticoccidial medications in turkey production include Amprolium (Amprol), Coyden (Clopidol), Halofuginone (Stenorol) (McDougald et al.,1986), Lasalocid (Avatec), Monensin (Coban), Zoalene (Zoamix) and Diclazuril (Clinacox) (Chapman et al.,2004). However, the overuse of these medications has contributed to the development of anticoccidial-resistant strains (Martins et al., 2022). Historical evidence underscores the critical issue of anticoccidial resistance in controlling coccidiosis, predominantly managed through chemical coccidiostats in feed (Shirley et al., 2005). This challenge is compounded by the continuous use of these medications with few strategies available to rest or rotate medications, which has facilitated the rise of resistant strains across various anticoccidial classes, including both chemical and ionophore compounds (Chapman, 1986, 1997; Rotibi et al., 1989; Bafundo and Jeffers 1990).

Anticoccidial resistance research in turkeys, tracing back to 1979 with the experiments conducted by Edgar and Flanagan demonstration of Stenorol efficacy, has evolved to uncover varying resistance patterns among *Eimeria* to medications like Coban, Avatec, and Diclazuril (Edgar and Flanagan, 1979; Jeffers and Bentley, 1980; Augustine et al., 1987; McDougald et al., 1991). Studies through 2007 to 2023, including work by Chapman, Rathinam, and Trujillo Peralta, have documented mixed responses to anticoccidials, highlighting the ongoing challenge of managing resistance in turkey populations (Chapman, 2007; Rathinam, 2009; Trujillo Peralta et al., 2023).

In general, the body of research from 1979 to 2023 illustrates the dynamic nature of anticoccidial resistance in turkeys. While significant steps have been made in understanding and managing this resistance, the studies collectively underscore the need for continued vigilance, innovative control strategies, and further research to sustainably manage coccidiosis in turkey populations. Therefore, the present research has been designed to address these critical issues comprehensively. The aim is to investigate the yearly effects of anticoccidial resistance in commercial turkey populations, specifically focusing on decreased levels of sensitivity to the most commonly used anticoccidial medications.

4.2 Materials and Methods

Study Design and Analysis of Anticoccidial Medication Resistance Trends

This research was designed to systematically investigate the efficacy of anticoccidial medications against *Eimeria* in commercial turkeys, with a primary focus on assessing resistance patterns and their impact on oocyst shedding. By integrating current laboratory data from anticoccidial sensitivity testing services between 2019 and 2023 at Ohio State University (see previous chapter) with historical findings, this study aims to provide a comprehensive overview of the evolution and current state of anticoccidial resistance in turkey populations. Through this approach, changes in anticoccidial resistance across decades can be observed, leveraging both newly acquired data and published reports from regions in the U.S. and Canada, to highlight the dynamics of anticoccidial efficacy against *Eimeria* infections.

Inclusion Criteria

For a study to be included in this research, it had to meet the following criteria:

- The paper must specifically address the resistance of *Eimeria* to anticoccidial medications in turkeys.
- The turkeys under investigation must be of a commercial breed, reflecting the common agricultural practices and environments where these medications are typically applied.
- The study must include an evaluation of oocyst shedding as a measure of anticoccidial efficacy, allowing for the assessment of the impact of anticoccidial resistance on the biological lifecycle of *Eimeria*.
- Only studies focusing on *Eimeria* that infect turkeys were considered, ensuring the relevance of the findings to the turkey industry.

Exclusion Criteria

Studies were excluded from this research under the following conditions:

- Articles that discussed anticoccidial resistance in poultry broadly, without specific focus on turkeys, were excluded to maintain the specificity of the research towards commercial turkey production.
- Research focusing on anticoccidial medications' resistance in domestic or wild turkeys not raised under commercial conditions was excluded to concentrate on the agricultural impact of anticoccidial resistance.

- Studies utilizing *Eimeria* isolates from chickens were not considered, as the goal was to examine the anticoccidial resistance patterns specifically in turkey-hosted *Eimeria*, acknowledging the host-specific dynamics of *Eimeria* infections and anticoccidial resistance mechanisms.

- Studies that relied on a single sample or a single *Eimeria* to declare an anticoccidial as 100% sensitive or 100% resistant, due to concerns about the reliability and representativeness of such findings. Additionally, studies were excluded if they did not categorize the level of anticoccidial resistance based on the number of shedding oocysts into categories such as sensitive, reduced sensitivity, and resistant. This criterion was established to ensure the inclusion of studies with robust and comprehensive data.

Search Strategy and Selection Process

A comprehensive literature search was conducted across several databases, including PubMed, Scopus, and Web of Science, to identify relevant studies published up to the present date. A combination of various words (Table 4.1) was used to ensure a broad capture of applicable research. The search was limited to English-language papers. Following the initial search, titles and abstracts were screened based on the inclusion and exclusion criteria. Full texts of potentially relevant studies were then reviewed for detailed assessment. The reference lists of selected papers were also examined to identify additional studies not captured in the initial database search.

Table 4.1: Keywords used to search relevant published data.

Key words
Anticoccidial medication resistance
Turkey *Eimeria*
Oocyst shedding
Coccidiostat resistance
Drug resistance in *Eimeria*
Anticoccidial resistance in *Eimeria*
Amprol resistance in Turkey *Eimeria*
Avatec resistance in Turkey *Eimeria*
Coban resistance in Turkey *Eimeria*
Coyden resistance in Turkey *Eimeria*
Stenorol resistance in Turkey *Eimeria*
Zoamix resistance in Turkey *Eimeria*

Data Extraction and Analysis

Data were extracted from each selected study regarding the study design, type of anticoccidial tested, *Eimeria* species investigated, methods of assessing anticoccidial resistance, and outcomes related to oocyst shedding. This information was accumulated to evaluate the current trends in anticoccidial resistance among commercial turkeys and the implications for managing coccidiosis in poultry agriculture.

Ethical Considerations

Given the nature of this research as a literature review and analysis, specific ethical approval was not required. However, the study was conducted following the ethical guidelines for systematic reviews and meta-analyses, ensuring respect for the original authors' work and conclusions.

Statistical analysis

The statistical analysis aimed to assess the effects of anticoccidial medications, time (year), and their interaction on the sensitivity and resistance patterns of *Eimeria* in commercial turkey farms. Due to the limited number of experiments available for inclusion in this study, a comprehensive time-by-medication interaction model could not be implemented. Consequently, the analysis was divided into two main components: the effect of the anticoccidial medications and the effect of time on medication sensitivity and resistance. To evaluate the impact of time and anticoccidial medication type on the sensitivity and resistance patterns, meta regression was applied using the SAS PROC GLM function, considering the paper (experiment) the random variable.

4.3 Results

Anticoccidial Medication Efficacy over time

As mentioned previously we were not able to evaluate the results on interaction of medication and time. Also, because not all the medications were used in all the experiments, and the imbalance in the use of the medications over time may bias the information on the medication resistance may be biased and is presented in Appendix C. There is decreased in sensitivity in 1.23% ($P < 0.01$; Table 4.2) per year since 1980 until present for all medications. There were no observed differences for reduce sensitivity or resistance overtime ($P = 0.32$ and $P = 0.13$, respectively; Table 4.2).

Table 4.2: Temporal Trends in Anticoccidial Sensitivity and Resistance among Commercial Turkeys.

Factor[1]			P values	
	Intercept[2]	Slope[3]	Intercept	slope
Sensitivity (%)	71.6 (14)	-1.23 (0.388)	< 0.01	0.01
Reduced Sensitivity (%)	18.8 (11.15)	0.36 (0.340)	0.17	0.32
Resistant (%)	12.9 (17.96)	0.82 (0.498)	0.51	0.13

[1] Represents the average of all the anticoccidial product tested and reported from 1980 to 2024.

[2] The slope represents the average value obtain from the medications used in the first study referenced in the current dissertation (1980)

[3] Annual changes observed for each Factor.

4.4 Discussion

The results in the current trial revealed a notable decrease regarding the sensitivity of commercial turkeys to anticoccidial medications from 1980 to 2024. The most significant finding was a statistically significant decrease in sensitivity, quantified as a 1.23% reduction indicating a gradual decline in the sensitivity of these medication per year over the assessed period. This decrease in medication sensitivity is critical, given the reliance on anticoccidial medications for managing coccidiosis in commercial turkeys.

The observed trend aligns with the Kadykalo et al. (2018) who discussed the universal presence of coccidiosis in poultry operations and the extensive economic losses it invites. They highlighted the increasing challenge of antimicrobial resistance, thereby underscoring the need for alternative control measures, including vaccination and improved management practices. Similarly, Sundar et al. (2017) specifically addressed the evolution of anticoccidial medication resistance, indicating that the extensive use of these medication, both therapeutically and prophylactically, has led to the development of resistance, which aligns with the findings of the current study.

Coccidia resistance against medications results from genetic mutations and the selective pressure from widespread medication use in poultry farming (Abbas et al., 2011; Attree et al., 2021). Continuous and prophylactic use of these medication creates an environment where only resistant strains of *Eimeria* can thrive and proliferate (Abbas et al., 2011). This situation is worsened by farm management practices that increase the risk of transmission of resistant strains, such as high stocking densities and suboptimal hygiene (Shamim et al., 2015). The genetic adaptability of *Eimeria* enables rapid evolution and spread of resistance, posing significant challenges to the poultry industry by compromising animal health and economic efficiency (Chapman and Blake, 2022). Addressing this issue requires alternative strategies beyond medication use, including vaccination and improved farm management practices.

In the current study the understanding of *Eimeria* sensitivity to anticoccidial medication in turkeys may be compromised due to uneven medication use and biasness in resistance assessment. The lack of consistent methodologies for measuring medication sensitivity, along with an emphasis on chicken *Eimeria* because to its commercial relevance, complicates tracking resistance trends, particularly in turkeys, where there is less research. This restricted focus contributes to a limited understanding of the resistance issue. The current focus on turkey *Eimeria* emphasizes the importance of standardizing research methods across all *Eimeria* species to reliably assess medication resistance and inform management tactics. Future research should strive for standardization and transparency to present a more accurate picture of resistance and improve management techniques.

Conclusion

Based on the findings in chapter 4 of this dissertation, the results offer a concerning decline in the sensitivity of anticoccidial medications against *Eimeria* in commercial turkey farms by comparing present data with previously reported findings. This poses significant challenges to poultry industry in effectively managing coccidiosis and maintaining flock health.

Pertaining to our current findings, it is concluded that the commercial turkey industry is continuously under a huge risk loss due to *Eimeria* challenges and effectively managing this challenge is crucial. The observed decrease in medication sensitivity underscores the urgent need for alternative control strategies such as vaccination and improved management practices. In addition to this, our results highlight the importance of continued surveillance and research efforts to monitor medication efficacy and resistance trends accurately. By addressing these challenges and finding alternative solutions, we can work towards mitigating the impact of coccidiosis on commercial turkey production and protecting the long-term sustainability of turkey industry.

Chapter 5: Conclusion

The collective findings of this dissertation illustrate a pressing issue in terms of *Eimeria* resistance against anticoccidial mediations in US Turkey industry. Coyden has emerged as more sensitive options followed by Stenorol against Turkey *Eimeria* under the current conditions, whereas the efficacy of Amprol, Avatec, Coban, and Zoamix, is compromised by elevated resistance levels. However, significant decline in the sensitivity of anticoccidials in turkeys from 1980 to 2024, highlighting an urgent need for new management strategies. These insights form a foundation for developing revised management strategies to mitigate the resistance crisis, ensuring the sustainability of commercial turkey production in the face of evolving coccidiosis challenges.

Bibliography

Abbas, R. Z., Iqbal, Z., and Khan, M. N. (2009). Studies on the development of resistance in coccidian field isolates against commonly used ionophores. In *Proceedings of the 5th International Poultry Conference* (pp. 1314-1322).

Abbas, R. Z., Iqbal, Z., Blake, D., Khan, M. N., and Saleemi, M. K. (2011). Anticoccidial drug resistance in fowl coccidia: the state of play revisited. *World's Poultry Science Journal*, *67*(2), 337-350.

Adang, L. K., and Isah, Z. (2016). Prevalence of *Eimeria* species in local breed Chicken in Gombe metropolis, Gombe State, Nigeria. *International Journal of Biological and Chemical Sciences*, 10(6), 2667-2676.

Agunos, A., Deckert, A., Léger, D., Gow, S., and Carson, C. (2019). Antimicrobials used for the therapy of necrotic enteritis and coccidiosis in broiler chickens and turkeys in Canada, farm surveillance results (2013–2017). *Avian diseases*, 63(3), 433-445.

Al-Badri, R., and Barta, J. R. (2012). The kinetics of oocyst shedding and sporulation in two immunologically distinct strains of *Eimeria* maxima, GS and M6. *Parasitology research*, 111, 1947-1952.

Alexandratos, N., and Bruinsma, J. (2012). World agriculture towards 2030/2050: the 2012 revision.

Al-Natour, M. Q., Suleiman, M. M., and Abo-Shehada, M. N. (2002). Flock-level prevalence of *Eimeria* species among broiler chicks in northern Jordan. *Preventive veterinary medicine*, 53(4), 305-310.

Al-Taee, A. F. M., and AlNeema, M. S. S. (2018). Incidence of *Eimeria* spp in chicken breeders in Al-Hamdania region. *Al-Anbar Journal of Veterinary Sciences*, 11(2).

Anthony, J. P., Fyfe, L., and Smith, H. (2005). Plant active components–a resource for antiparasitic agents? *Trends in parasitology*, *21*(10), 462-468.

Arabkhazaeli, F., Modrisanei, M., Nabian, S., Mansoori, B., and Madani, A. (2013). Evaluating the resistance of *Eimeria spp.* field isolates to anticoccidial drugs using three different indices. *Iranian journal of parasitology*, 8(2), 234.

Arczewska-Włosek, A., Świątkiewicz, S., Ognik, K., and Józefiak, D. (2022). Effects of a dietary multi-strain probiotic and vaccination with a live anticoccidial vaccine on growth performance and haematological, biochemical and redox status indicators of broiler Chicken. *Animals*, *12*(24), 3489.

Asaaga, S. A., Ogbe, E. P., and Owoya, A. H. Prevalence of *Eimeria* oocysts among broiler and layer flocks in selected poultry farms in Makurdi, Benue State, Nigeria. *Journal of Bioscience and Agriculture Research*, 29(02), 2450-2455.

Attias, M., Teixeira, D. E., Benchimol, M., Vommaro, R. C., Crepaldi, P. H., and De Souza, W. (2020). The life cycle of Toxoplasma gondii reviewed using animations. *Parasites and vectors*, *13*, 1-13.

Attree, E., Sanchez-Arsuaga, G., Jones, M., Xia, D., Marugan-Hernandez, V., Blake, D., and Tomley, F. (2021). Controlling the causative agents of coccidiosis in domestic chickens; an eye on the past and considerations for the future. *CABI Agriculture and Bioscience*, *2*, 1-16.

Augustine, P. C. (2001). Cell: sporozoite interactions and invasion by apicomplexan parasites of the genus *Eimeria*. *International Journal for Parasitology*, *31*(1), 1-8.

Augustine, P. C., Smith II, C. K., Danforth, H. D., and Ruff, M. D. (1987). Effect of ionophorous anticoccidials on invasion and development of: comparison of sensitive and resistant isolates and correlation with drug uptake. *Poultry Science*, *66*(6), 960-965.

Bafundo KW, Jeffers TK (1990). Selection for resistance to monensin, nicarbazin, and the monensin plus nicarbazin combinations. *Poultry Science*, *69*:1485–1490

Ball, S. J., Pittilo, R. M., Norton, C. C., & Joyner, L. P. (1987). Ultrastructural studies of the effects of amprolium and dinitolmide on *Eimeria* acervulina macrogametes. *Parasitology Research*, *73*, 293-297.

Barnes, H. J., Vaillancourt, J. P., and Gross, W. P. (2003). Colibacillosis In “Disease of Poultry” Ed. By YM Saif, HJ Barnes, AM Fadly, JR Glisson, LR McDougald, DE Swayne, 631-646.

Bauer, J., Kaske, M., Oehm, A., and Schnyder, M. (2023). A pilot study for the isolation of *Eimeria* spp. oocysts from environmental straw samples in comparison with individual faecal examination of fattening calves. *Parasitology Research*, 1-9.

Bawm, S., Win, S.Y., Soe, N.C., Thaw, Y.N., Hmoon, M.M., Htun, L.L., Nakao, R. and Katakura, K. (2021). Microscopic and molecular detection of *Eimeria* maxima and *Eimeria* praecox naturally infected in free-range village Chicken of Myanmar. *Acta Parasitologica*, 66, 1074-1078.

Bi, F., Hao, Z., Sun, P., Yu, Y., Suo, X., and Liu, X. (2018). Studies on anticoccidial drugs and drug resistance of coccidiosis in chickens. *Acta Parasitologica et Medica Entomologica Sinica*, *25*(4), 242-253.

Blake, D.P., Knox, J., Dehaeck, B., Huntington, B., Rathinam, T., Ravipati, V., Ayoade, S., Gilbert, W., Adebambo, A.O., Jatau, I.D. and Raman, M. (2020). Re-calculating the cost of coccidiosis in Chicken. *Veterinary Research*, *51*, 1-14.

Blake, D. P., Pastor-Fernández, I., Nolan, M. J., and Tomley, F. M. (2017). Recombinant anticoccidial vaccines-a cup half full. *Infection, Genetics and Evolution*, *55*, 358-365.

Braunius, W. W. (1988). Epidemiology of *Eimeria* in broiler chicks as influenced by anticoccidial agents. *Tijdschrift voor diergeneeskunde, 113*(3), 123-131.

Bucek, O. C. (1969). The Effect of Coyden Coccidiostat (Containing Clopidol) on Pullets and Laying Hens. *Poultry science*, *48*(6), 2173-2177.

Carrisosa, M., Jin, S., McCrea, B. A., Macklin, K. S., Dormitorio, T., and Hauck, R. (2021). Prevalence of select intestinal parasites in Alabama backyard poultry flocks. *Animals*, *11*(4), 939.

Chapman, H. D., & Rathinam, T. (2022). Focused review: the role of drug combinations for the control of coccidiosis in commercially reared chickens. *International Journal for Parasitology: Drugs and Drug Resistance*, *18*, 32-42.

Chapman HD. (1986). Drug resistance in coccidia: recent research. In L.R. McDougald, L.P. Joyner and P.L. Long (Eds.), Proceedings of the Georgia Coccidiosis Conference. 330 -341 Georgia, USA.

Chapman, H. D. (1997). Biochemical, genetic and applied aspects of drug resistance in *Eimeria* parasites of the fowl. *Avian pathology*, *26*(2), 221-244.

Chapman, H. D. (1982). The use of enzyme electrophoresis for the identification of the species of *Eimeria* present in field isolates of coccidia. *Parasitology*, *85*(3), 437-442.

Chapman, H. D. (1984). Drug resistance in avian coccidia (a review). *Veterinary Parasitology*, 15, 11-27.

Chapman, H. D. (1985). Drug resistance in coccidia: recent research. Research in Avian Coccidiosis, 330-347.

Chapman, H. D. (1993). Resistance to anticoccidial drugs in fowl. *Parasitology Today*, 9, 159-162.

Chapman, H. D. (1994). A review of the biological activity of the anticoccidial drug nicarbazin and its application for the control of coccidiosis in poultry. *Poultry Science Reviews (United Kingdom)*.

Chapman, H. D. (1994). Sensitivity of field isolates of *Eimeria* to monensin following the use of a coccidiosis vaccine in broiler chickens. *Poultry Science*, *73*(3), 476-478.

Chapman, H. D. (1997). Biochemical, genetic and applied aspects of drug resistance in *Eimeria* parasites of the fowl. *Avian pathology*, *26*(2), 221-244.

Chapman, H. D. (2001). Use of anticoccidial drugs in broiler Chicken in the USA: analysis for the years 1995 to 1999. *Poultry Science*, *80*(5), 572-580.

Chapman, H. D. (2008). Coccidiosis in the turkey. *Avian pathology*, *37*(3), 205-223.

Chapman, H. D. (2009). A landmark contribution to poultry science—prophylactic control of coccidiosis in poultry. *Poultry science*, *88*(4), 813-815.

Chapman, H. D., and Jeffers, T. K. (2014). Vaccination of Chicken against coccidiosis ameliorates drug resistance in commercial poultry production. *International Journal of Parasitology: Drugs and Drug Resistance*, 4, 214-217.

Chapman, H. D., and Jeffers, T. K. (2015). Restoration of sensitivity to salinomycin in *Eimeria* following 5 flocks of broiler Chicken reared in floor-pens using drug programs and vaccination to control coccidiosis. *Poultry Science*, *94*(5), 943-946.

Chapman, H. D., and Saleh, E. (1999). Effects of different concentrations of monensin and monensin withdrawal upon the control of coccidiosis in the turkey. *Poultry science*, *78*(1), 50-56.

Chapman, H.D., Barta, J.R., Blake, D., Gruber, A., Jenkins, M., Smith, N.C., Suo, X. and Tomley, F.M. (2013). A selective review of advances in coccidiosis research. *Advances in parasitology*, *83*, 93-171.

Chapman, H. D., and Blake, D. P. (2022). Genetic selection of *Eimeria* parasites in the chicken for improvement of poultry health: implications for drug resistance and live vaccine development. *Avian Pathology*, *51*(6), 521-534.

Chapman, H. D., Matsler, P. L., and Chapman, M. E. (2004). Control of coccidiosis in turkeys with diclazuril and monensin: effects upon performance and development of immunity to . *Avian diseases, 48*(3), 631-634.

Chapman, H., Cherry, T. E., Danforth, H. D., Richards, G., Shirley, M. W., and Williams, R. B. (2002). Sustainable coccidiosis control in poultry production: the role of live vaccines. *International Journal for Parasitology*, *32*(5), 617-629.

Chapman, H.D. (1997) Biochemical, Genetic, And Applied Aspects Of Drug Resistance In *Eimeria* Parasites Of The Fowl. *Avian Pathology 26*: 221-244.

Chawla, J., Oberstaller, J., and Adams, J. H. (2021). Targeting gametocytes of the malaria parasite Plasmodium falciparum in a functional genomics era: next steps. *Pathogens*, *10*(3), 346.

Christaki, E., Florou-Paneri, P., Giannenea, I., Papzahariadou, M., Botsoglou, N. A., and Spais, A. S. B. (2004). Effect of a mixture of herbal extracts on broiler Chicken infected with *Eimeria* tenella. *Animal Research*, *53*, 137-144.

Clark, S. R. (2019). Future of coccidiosis management in turkeys. Devenish Nutrition.

Clark, S. R., and Pyle, D. (2009). Current health and industry issues facing the turkey industry. In *Annual Proceedings of the US Animal Health Association Meeting*. San Diego, CA: Transmissible Diseases of Poultry and Other Avian Species Committee.

Clarkson, M.J. (1958). Life history and pathogenicity of *Eimeria adenoides* Moore and Brown, 1951, in the turkey poult. *Parasitology*, *48*, 7088.

Cornell, K. A. (2020). *Epizootiology of coccidia in organic poultry: Management, risks, and future research* (Doctoral dissertation, Washington State University).

Cuomo, M. J., Noel, L. B., and White, D. B. (2009). Diagnosing medical parasites: a public health officers guide to assisting laboratory and medical officers. *US, OH: Air Education and Training Command, RANDOLPH AFB TX.*

Dalloul, R. A., and Lillehoj, H. S. (2005). Recent advances in immunomodulation and vaccination strategies against coccidiosis. *Avian diseases*, *49*(1), 1-8.

Dalloul, R. A., and Lillehoj, H. S. (2006). Poultry coccidiosis: recent advancements in control measures and vaccine development. *Expert review of vaccines*, *5*(1), 143-163.

Dalloul, R. A., Bliss, T. W., Hong, Y. H., Ben-Chouikha, I., Park, D. W., Keeler, C. L., and Lillehoj, H. S. (2007). Unique responses of the avian macrophage to different species of *Eimeria*. *Molecular immunology*, *44*(4), 558-566.

Debbou-Iouknane, N., Benbarek, H., and Ayad, A. (2018). Prevalence and aetiology of coccidiosis in broiler Chicken in Bejaia province, Algeria. *Onderstepoort Journal of Veterinary Research*, *85*(1), 1-6.

Devenish Nutrition. (2023). Future of Coccidiosis Management in Turkeys. Retrieved August 25, 2023, from https://us.devenishnutrition.com/press-releases/153/future-of-coccidiosis-management-in-turkeys.

Dubey, J. P. (2018). A review of coccidiosis in South American camelids. *Parasitology research*, *117*, 1999-2013.

Dubey, J. P., Ferreira, L. R., Martins, J., and Jones, J. L. (2011). Sporulation and survival of Toxoplasma gondii oocysts in different types of commercial cat litter. *Journal of Parasitology*, *97*(5), 751-754.

Dubey, J. P., Lindsay, D. S., Jenkins, M. C., and Bauer, C. (2019). Biology of intestinal coccidia. In *Coccidiosis in livestock, poultry, companion animals, and humans* (pp. 1-36). CRC Press.

Durairaj, V., Veen, R. V., and Clark, S. (2023). Predominant *Eimeria* Species in Turkeys: Diagnosis and Control. *International Animal Health Journal*, *10*(2).

Duszynski, D. W., Kvičerová, J., and Seville, R. S. (2018). *The biology and identification of the Coccidia (Apicomplexa) of carnivores of the world*. Academic Press.

EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP), Bampidis, V., Azimonti, G., Bastos, M.D.L., Christensen, H., Dusemund, B., Fašmon Durjava, M., Kouba, M., López-Alonso, M., López Puente, S. and Marcon, F. (2022). Safety of a feed additive consisting of halofuginone hydrobromide (STENOROL®) for chickens for fattening and turkeys (Huvepharma NV). *EFSA Journal*, *20*(12), e07716.

Fatoba, A. J., and Adeleke, M. A. (2018). Diagnosis and control of chicken coccidiosis: a recent update. *Journal of Parasitic Diseases*, *42*, 483-493.

Fatoba, A. J., Zishiri, O. T., Blake, D. P., Peters, S. O., Lebepe, J., Mukaratirwa, S., and Adeleke, M. A. (2020). Study on the prevalence and genetic diversity of *Eimeria* species from broilers and free-range Chicken in KwaZulu-Natal province, South Africa. *Onderstepoort Journal of Veterinary Research*, *87*(1), 1-10.

Flores, R.A., Nguyen, B.T., Cammayo, P.L.T., Võ, T.C., Naw, H., Kim, S., Kim, W.H., Na, B.K. and Min, W. (2022). Epidemiological investigation and drug resistance of *Eimeria* species in Korean chicken farms. *BMC Veterinary Research*, *18*(1), 277.

Gadde, U. D., Rathinam, T., Finklin, M. N., and Chapman, H. D. (2020). Pathology caused by three species of *Eimeria* that infect the turkey with a description of a scoring system for intestinal lesions. *Avian pathology*, *49*(1), 80-86.

Geng, T., Ye, C., Lei, Z., Shen, B., Fang, R., Hu, M., Zhao, J. and Zhou, Y. (2021). Prevalence of *Eimeria* parasites in the Hubei and Henan provinces of China. *Parasitology Research*, *120*, 655-663.

George, A. G., and Sabu, L. (2002). Evaluation of amprolium hydrochloride, monensin and salinomycin as coccidiostats in broiler chicken. *Indian Journal of Poultry Science*, *37*(2), 159-162.

Gerhold, R. W. (2016). Overview of coccidiosis in poultry. *Merck Veterinary Manual. 11th ed Merck & Co, Inc*.

Gilbert, W., Bellet, C., Blake, D. P., Tomley, F. M., and Rushton, J. (2020). Revisiting the economic impacts of *Eimeria* and its control in European intensive broiler systems with a recursive modeling approach. Frontiers in Veterinary Science, 7, 558182.

Godwin, R. M., and Morgan, J. A. (2015). A molecular survey of *Eimeria* in Chicken across Australia. *Veterinary parasitology*, *214*(1-2), 16-21.

Greif, G., Harder, A., and Haberkorn, A. (2001). Chemotherapeutic approaches to protozoa: Coccidiae--current level of knowledge and outlook. *Parasitology Research*, *87*(11), 973-975.

Györke, A., Kalmár, Z., Pop, L. M., and Şuteu, O. L. (2016). The economic impact of infection with *Eimeria* spp. in broiler farms from Romania. *Revista Brasileira de Zootecnia*, *45*, 273-280.

Györke, A., Pop, L., and Cozma, V. (2013). Prevalence and distribution of *Eimeria* species in broiler chicken farms of different capacities. *Parasite*, 20.

Hadipour, M. M., Olyaie, A., Naderi, M., Azad, F., and Nekouie, O. (2011). Prevalence of *Eimeria* species in scavenging native Chicken of Shiraz, Iran. *African Journal of Microbiology Research*, *5*(20), 3296-3299.

Hamidinejat, H., Shapouri, M. S., Mayahi, M., and Borujeni, M. P. (2010). Characterization of *Eimeria* species in commercial broilers by PCR based on ITS1 regions of rDNA. *Iranian journal of parasitology*, *5*(4), 48.

Harper, J. K., and Kime, L. (2021). Small Flock Turkey Production. Penn State Extension. Retrieved from https://extension.psu.edu/small-flock-turkey-production

Hawkins, P.A. (1952). Coccidiosis in Turkeys Technical Bulletin 226. East Lansing, MI: Michigan State College Agricultural Experiment Station

Hein, H. (1974). *Eimeria* brunetti: Pathogenic effects in young Chicken. *Experimental Parasitology*, *36*(3), 333-341.

Henken, A. M., Ploeger, H. W., Graat, E. A. M., and Carpenter, T. E. (1994). Description for a simulation model for the population dynamics of *Eimeria* acervulina infection in broilers. *Parasitology*, 108, 503-512.

Huang, B., Zhao, Q. P., Wu, X. Z., Chen, Z. G., Chen, Y. J., Shi, T. W., and Ye, M. Z. (2001). Studies on drug resistance in field isolates of coccidia from chickens in Shanghai. *Chin. J. Vet. Parasitol*, *9*, 1-7.

Huang, Y., Ruan, X., Li, L., and Zeng, M. (2017). Prevalence of *Eimeria* species in domestic Chicken in Anhui province, China. *Journal of Parasitic Diseases*, *41*, 1014-1019.

Imai, R. K. (2018). *Diversity and cross-immunity of Eimeria species infecting turkeys in commercial flocks in Canada* (Doctoral dissertation, University of Guelph).

Imai, R. K., and Barta, J. R. (2019). Distribution and abundance of *Eimeria* species in commercial turkey flocks across Canada. *The Canadian veterinary journal*, *60*(2), 153.

Iqbal, M. A., and Begum, S. (2010). Prevalence of coccidiosis in broiler chicken. *International Journal of Animal and Fishery Sciences*, *3*(1), 270-273.

James, S. (1980) Thiamine uptake in isolated schizonts of *Eimeria* tenella and the inhibitory effects of amprolium. *Parasitology, 80*: 313-322.

Jeffers, T. K. (1974). *Eimeria* acervulina and E. maxima: incidence and anticoccidial drug resistance of isolants in major broiler-producing areas. *Avian diseases*, 331-342.

Jeffers, T. K., and Bentley, E. J. (1980). Monensin sensitivity of recent field isolates of turkey coccidia. *Poultry Science*, *59*(8), 1722-1730.

Jeffers, T. K., and Challey, J. R. (1973). Collateral sensitivity to 4-hydroxyquinolines in acervulina strains resistant to meticlorpindol. *The Journal of Parasitology*, 624-630.

Jeffers, T.K. (1989) Anticoccidial drug resistance: a review with emphasis on the polyether ionophores, in: YVORE, P. (Ed.) Coccidia, and intestinal coccidiomorphs, Vth International Coccidiosis Conference, pp. 295-308 (Tours, France, Paris: INRA Publications).

Jenkins, M. C., Cline, J., Parker, C., O'Brien, C., Burleson, M., and Schaeffer, J. (2023). Administering *Eimeria* maxima oocysts through drinking water improves coccidiosis vaccine uptake in broiler Chicken. *Journal of Applied Poultry Research*, *32*(1), 100312.

Joyner, L. P., and Norton, C. C. (1969). A comparison of two laboratory strains of *Eimeria* tenella. *Parasitology*, *59*(4), 907-913.

Kadykalo, S., Roberts, T., Thompson, M., Wilson, J., Lang, M., and Espeisse, O. (2018). The value of anticoccidials for sustainable global poultry production. *International Journal of Antimicrobial Agents*, *51*(3), 304-310.

Khan, M. Q., Irshad, H., Anjum, R., Jahangir, M., and Nasir, U. (2006). Eimeriosis in poultry of Rawalpindi/Islamabad area. *Pakistan Veterinary Journal*, *26*(2), 85-87.

Kheysin, Y. M. (2013). *Life cycles of coccidia of domestic animals*. Elsevier.

Khursheed, A., Yadav, A., Sofi, O.M.U.D., Kushwaha, A., Yadav, V., Rafiqi, S.I., Godara, R. and Katoch, R. (2022). Prevalence and molecular characterization of *Eimeria* species affecting backyard poultry of Jammu region, North India. *Tropical Animal Health and Production*, *54*(5), 296.

Kitandu, A., and Juranova, R. (2006). Progress in control measures for chicken coccidiosis. *Acta Veterinaria Brno*, 75, 265-276.

Lakkundi, J., Jagannath, M. S., Vijayasarathi, S. K., and Souza, P. E. (2002). Histopathological evaluation of anticoccidial activity of toltrazuril and amprolium in experimental tenella infection in chicken. *The Indian Journal of Animal Sciences*, *72*(5).

Lan, L. H., Sun, B. B., Zuo, B. X. Z., Chen, X. Q., and Du, A. F. (2017). Prevalence and drug resistance of avian *Eimeria* species in broiler chicken farms of Zhejiang province, China. *Poultry Science*, *96*(7), 2104-2109.

Lee, B.H., Kim, W.H., Jeong, J., Yoo, J., Kwon, Y.K., Jung, B.Y., Kwon, J.H., Lillehoj, H.S. and Min, W. (2010). Prevalence and cross-immunity of *Eimeria* species on Korean chicken farms. *Journal of Veterinary Medical Science*, *72*(8), 985-989.

Li, G. Q., Kanu, S., Xiang, F. Y., Xiao, S. M., Zhang, L., Chen, H. W., and Ye, H. J. (2004). Isolation and selection of ionophore-tolerant *Eimeria* precocious lines: E. tenella, E. maxima and E. acervulina. *Veterinary Parasitology*, *119*(4), 261-276.

Li, W., Wang, M., Chen, Y., Chen, C., Liu, X., Sun, X., Jing, C., Xu, L., Yan, R., Li, X. and Song, X. (2020). EtMIC3 and its receptors BAG1 and ENDOUL are essential for site-specific invasion of *Eimeria* tenella in Chicken. *Veterinary Research*, *51*, 1-15.

Lilić, S., Ilić, T., and Dimitrijević, S. (2009). Coccidiosis in poultry industry. *Scientific journal" Meat Technology"*, *50*(1-2), 90-98.

Liu, Q., Liu, X., Zhao, X., Zhu, X. Q., and Suo, X. (2023). Live attenuated anticoccidial vaccines for chickens. *Trends in Parasitology*.

López-Osorio, S., Chaparro-Gutiérrez, J. J., and Gómez-Osorio, L. M. (2020). Overview of poultry *Eimeria* life cycle and host-parasite interactions. *Frontiers in veterinary science*, *7*, 384.

Luu, L., Bettridge, J., Christley, R.M., Melese, K., Blake, D., Dessie, T., Wigley, P., Desta, T.T., Hanotte, O., Kaiser, P. and Terfa, Z.G. (2013). Prevalence and molecular characterisation of *Eimeria* species in Ethiopian village Chicken. *BMC Veterinary Research*, *9*, 1-7.

Martins, R. R., Silva, L. J., Pereira, A. M., Esteves, A., Duarte, S. C., and Pena, A. (2022). Coccidiostats and poultry: A comprehensive review and current legislation. *Foods*, *11*(18), 2738.

Mathis, G. F., and Broussard, C. (2006). Increased level of *Eimeria* sensitivity to diclazuril after using a live coccidial vaccine. *Avian diseases*, *50*(3), 321-324.

Mathis, G., Van-Heerden, K., and Lumpkins, B. (2021). Anticoccidial drug sensitivity of *Eimeria* contained in live coccidia vaccines of broilers, breeders, and turkeys. *Avian Diseases*, *65*(3), 358-363.

Matsubayashi, M., Shibahara, T., Matsuo, T., Hatabu, T., Yamagishi, J., Sasai, K., and Isobe, T. (2020). Morphological and molecular identification of *Eimeria* spp. in breeding chicken farms of Japan. *Journal of Veterinary Medical Science*, *82*(5), 516-519.

McDougald, L. R. (1986). Protozoan Infections of Domestic Animals: Coccidian and Related Infections. In Chemotherapy of Parasitic Diseases (pp. 159-170). Boston, MA: Springer US.

McDougald, L. R., and Fitz-Coy, S. H. (2008). Coccidiosis (Chapter 28-Protozoal Infections). *Diseasesof Poultry (12th Ed.). Saif YM et al. (ed.). Wiley-Blackwell Publishing, Ames, Iowa*.

McDougald, L. R., Fuller, L., and Mattiello, R. (1997). A survey of coccidia on 43 poultry farms in Argentina. *Avian Diseases*, 923-929.

McDougald, L. R., Mathis, G. F., and Seibert, B. P. (1991). Anticoccidial efficacy of diclazuril against recent field isolates of from turkey farms in the US. *Avian Diseases*, 863-868.

Mcdougald, L. R., Mathis, G. F., Schwartz, J., Quarles, C. L., Kennedy, T., and Grant, R. J. (1986). Anticoccidial efficacy of halofuginone in turkeys reared to market weight. *Poultry Science*, *65*(9), 1664-1670.

McDougald, L.R. and W.M. Reid, 1994. Coccidiosis. In: Calnek, B.W. (ed.), Diseases of Poultry, 9th edition. Affiliated East West Press Pvt. Ltd., New Delhi, India

McLoughlin, D. K. (1970). Coccidiosis: Experimental analysis of drug resistance. Experimental Parasitology, 28(1), 129-136.

Mesa-Pineda, C., Navarro-Ruíz, J. L., López-Osorio, S., Chaparro-Gutiérrez, J. J., and Gómez-Osorio, L. M. (2021). Chicken coccidiosis: from the parasite lifecycle to control of the disease. *Frontiers in Veterinary Science*, *8*, 787653.

Miglani, G. S., Sharma, A. K., and Singh, S. (2002). Mechanisms of co-evolution in host-parasite system–a fresh look. *Jour Pl Sci Res*, *13*, 1-8.

Milbradt, E.L., Mendes, A.A., Ferreira, J.G., Paz, I.A., Martins, M.B., Sanfelice, C., Fernandes, B.C. and Okamoto, A.S. (2014). Use of live oocyst vaccine in the control of turkey coccidiosis: effect on performance and intestinal morphology. *Journal of Applied Poultry Research*, *23*(2), 204-211.

Mio, J. B., Mohamed, M. O. S., Ahmed, Z. A., Salah, H. M., Abdulkadir, B. A., Hussein, A. H., and Afrah, O. H. (2022). Prevalence of Poultry Coccidiosis and Associated Risk Factors in Intensive Farm and Individual Small Holder Poultry Farm in Benadir Region, Somalia. *Integrated Journal for Research in Arts and Humanities*, *2*(4), 71-76.

Moore, E. N., and Brown, J. A. (1951). A new coccidium pathogenic for turkeys, *Eimeria adenoides* n. sp. (Protozoa:Eimeriidae). *Cornell Veterinarian*. 41, 125-136.

Moore, E. N., and Brown, J.A. (1952). A new coccidium of turkeys, *Eimeria innocua* n. sp. (Protozoa: Eimeriidae). *Cornell Veterinarian*. 42, 395-402.

Moore, E.N. (1954). Species of coccidia affecting turkeys. Proceedings of the American Veterinary Medical Association, 91, 300303.

Moore, E.N., Brown, J.A. and Carter, R.D. (1954). A new coccidium of turkeys, *Eimeria* subrotunda N. sp. (Protozoa: Eimeriidae). *Poultry Science*, *33*, 925929.

Moraes, J.C., França, M., Sartor, A.A., Bellato, V., de Moura, A.B., Magalhães, M.D.L.B., de Souza, A.P. and Miletti, L.C. (2015). Prevalence of *Eimeria* spp. in broilers by multiplex PCR in the southern region of Brazil on two hundred and fifty farms. *Avian Diseases*, *59*(2), 277-281.

Muazu, A., Masdooq, A.A., Ngbede, J., Salihu, A.E., Haruna, G., Habu, A.K., Sati, M.N. and Jamilu, H. (2008). Prevalence and identification of species of *Eimeria* causing coccidiosis in poultry within Vom, Plateau State, Nigeria. *international journal of Poultry Science*, *7*(9), 917-918.

Muthamilselvan, T., Kuo, T. F., Wu, Y. C., and Yang, W. C. (2016). Herbal remedies for coccidiosis control: A review of plants, compounds and anticoccidial actions. *Evidence-Based Complementary and Alternative Medicine*, 1-19.

Naidoo, V., McGaw, L. J., Bisschop, S. P., Duncan, N., and Eloff, J. N. (2008). The value of plant extracts with antioxidant activity in attenuating coccidiosis in broiler Chicken. *Veterinary Parasitology*, 153, 214-219.

National Turkey Federation (2015). https://www.eatturkey.org/

National Turkey Federation. (n.d.). Industry. Retrieved June 26, 2023, from https://www.eatturkey.org/industry/

Nematollahi, A., Moghaddam, G., & Pourabad, R. F. (2009). Prevalence of *Eimeria* species among broiler chicks in Tabriz (Northwest of Iran). *Mun. Ent. Zool*, *4*(1), 53-58.

Nilsson, O., Greko, C., Bengtsson, B., and Englund, S. (2012). Genetic diversity among VRE isolates from Swedish broilers with the coincidental finding of transferrable decreased susceptibility to narasin. *Journal of applied microbiology*, *112*(4), 716-722.

Noack, S., Chapman, H. D., and Selzer, P. M. (2019). Anticoccidial drugs of the livestock industry. *Parasitology research*, *118*, 2009-2026.

Ogedengbe, J. D., Hunter, D. B., and Barta, J. R. (2011). Molecular identification of *Eimeria* species infecting market-age meat Chicken in commercial flocks in Ontario. *Veterinary parasitology*, *178*(3-4), 350-354.

Ojimelukwe, A. E., Emedhem, D. E., Agu, G. O., Nduka, F. O., and Abah, A. E. (2018). Populations of *Eimeria* tenella express resistance to commonly used anticoccidial drugs in southern Nigeria. *International Journal of Veterinary Science and Medicine*, *6*(2), 192-200.

Okwuonu, E. S., Eneh, C. M., Elijah, E. N., Nnaji, F. N., Andong, F. A., Ani, C. P., and Nwosu, C. O. (2021). Prevalence of *Eimeria* infection and risk factors associated with domestic Chicken raised in Nsukka LGA, Enugu State, Southeast Nigeria. *Nigerian Journal of Parasitology*, 42(2).

Ola-Fadunsin, S. D., Uwabujo, P. I., Sanda, I. M., Hussain, K., Ganiyu, I. A., Rabiu, M., and Balogun, R. B. (2019). Cross-sectional study of *Eimeria* species of poultry in Kwara State, North-Central Nigeria. *Journal of Parasitic Diseases*, 43, 87-95.

Pawestri, W., Nuraini, D. M., and Andityas, M. (2020). The estimation of economic losses due to coccidiosis in broiler Chicken in Central Java, Indonesia. In IOP Conference Series: Earth and Environmental Science (Vol. 411, No. 1, p. 012030). IOP Publishing.

Peek, H. W., and Landman, W. J. M (2010). Coccidiosis in poultry: anticoccidial products and alternative prevention strategies (a review). *Resistance to anticoccidial drugs: alternative strategies to control coccidiosis in broilers*, 5.

Peek, H. W., and Landman, W. J. M. (2003). Resistance to anticoccidial drugs of Dutch avian spp. field isolates originating from 1996, 1999 and 2001. *Avian Pathology*, *32*(4), 391-401.

Peek, H. W., and Landman, W. J. M. (2006). Higher incidence of *Eimeria spp.* field isolates sensitive for diclazuril and monensin associated with the use of live coccidiosis vaccination with Paracox™-5 in broiler farms. *Avian Diseases*, *50*(3), 434-439.

Peek, H. W., and Landman, W. J. M. (2011). Coccidiosis in poultry: anticoccidial products, vaccines and other prevention strategies. *Veterinary quarterly*, *31*(3), 143-161.

Peng, X., Zhang, J., Wei, W., Wu, H., and Xie, M. (2000). Resistance of tenella isolates from Guangdong province of China to 3 coccidiostats. *Chinese Journal of Veterinary Science*, *20*(1), 48-50.

Pines, M., and Spector, I. (2015). Halofuginone—the multifaceted molecule. *Molecules*, *20*(1), 573-594.

Qi, Z. (2003). Study On Drug-Resistance to Seven Anticoccidia Drugs Of Chicken Coccidian From Qingyuan, Guangdong.

Quiroz-Castaneda, R. E., and Dantan-Gonzalez, E. (2015). Control of Avian Coccidiosis: Future and Present Natural Alternatives. *Biomed Research International*. https://doi.org/10.1155/2015/430610

Radu, J. (2008). Immunity develops earlier in turkeys vaccinated for coccidiosis. North American Edition (2). Special report of the 57th WPDC 2008.

Rampin, T., Manarolla, G., Recordati, C., and Sironi, G. (2006). Caecal coccidiosis in commercial male turkeys. *Italian Journal of Animal Science*, *5*(3), 315-317.

Rao, P. V., Raman, M., Dhinakarraj, G., Basith, S. A., and Gomathinayagam, S. (2012). Speciation of poultry *Eimeria* by morphometry and SCAR PCR in Southern India. *Indian Journal of Animal Sciences*, *82*(8), 805-811.

Rathinam, T., and Chapman, H. D. (2009). Sensitivity of isolates of *Eimeria* from turkey flocks to the anticoccidial drugs amprolium, clopidol, diclazuril, and monensin. *Avian diseases*, *53*(3), 405-408.

Répérant, J. M., Thomas-Hénaff, M., Benoit, C., Le Bihannic, P., and Eterradossi, N. (2021). The impact of maturity on the ability of *Eimeria* acervulina and *Eimeria* meleagrimitis oocysts to sporulate. *Parasite*, *28*.

Reyna, P. S., McDougald, L. R., and Mathis, G. F. (1983). Survival of coccidia in poultry litter and reservoirs of infection. *Avian Diseases*, 464-473.

Rogers, E.F. (1962) Thiamine antagonists. Annals of the New York Academy of Science 98: 412-429.

Rotibi A, Mcdougald LR, Solis J. (1989). Response of 21 Canadian field isolates of chicken coccidia to commercial anticoccidial drugs. *Avian Diseases*, *33*:365–367

Ruff, M. D., Schorr, L., Davidson, W. R., and Nettles, V. F. (1988). Prevalence and identity of coccidia in pen-raised wild turkeys. *Journal of wildlife diseases*, *24*(4), 711-714.

Sandu, D. (2023). New challenges in coccidiosis control. Retrieved from https://www.alltech.com/blog/new-challenges-coccidiosis-control on 2024, February 20

Shahbandeh, M. (2023). Poultry industry in the US - statistics and facts. Statista. Retrieved February 28, 2024, from https://www.statista.com/topics/6263/poultry-industry-in-the-united-states/#topicOverview

Shahraki, F., Shariati-Sharifi, F., Nabavi, R., and Jamshidian, A. (2018). Coccidiosis in Sistan: the prevalence of *Eimeria* species in native chicken and its histopathological changes. *Comparative Clinical Pathology*, *27*, 1537-1543.

Shamim, A., ul Hassan, M., Yousaf, A., Iqbal, M. F., Zafar, M. A., Siddique, R. M., and Abubakar, M. (2015). Occurrence and identification of Emeria species in broiler rearing under traditional system. *Journal of Animal Science and Technology*, *57*, 1-4.

Sherkov, S. (1977). Effect of feeding egg whites and thiamine on coccidiosis caused by tenella in chicks.

Shirley, M. W., Smith, A. L., & Tomley, F. M. (2005). The biology of avian *Eimeria* with an emphasis on their control by vaccination. *Advances in parasitology*, *60*, 285-330.

Shivaramaiah, C., Barta, J. R., Hernandez-Velasco, X., Téllez, G., and Hargis, B. M. (2014). Coccidiosis: recent advancements in the immunobiology of *Eimeria* species, preventive measures, and the importance of vaccination as a control tool against these Apicomplexan parasites. *Veterinary Medicine: Research and Reports*, 23-34.

Snyder, R. (2021). Coccidiosis in commercial broiler chickens: Improving management of *Eimeria* species using live-vaccination or anticoccidial medication and developing and applying quantitative species-specific molecular assays (Doctoral dissertation, University of Guelph).

Snyder, R.P., Guerin, M.T., Hargis, B.M., Kruth, P.S., Page, G., Rejman, E., Rotolo, J.L., Sears, W., Zeldenrust, E.G., Whale, J. and Barta, J.R. (2021). Restoration of anticoccidial sensitivity to a commercial broiler chicken facility in Canada. *Poultry Science*, *100*(2), 663-674.

Stevens, D. D. (1998). Coccidiosis: Encyclopedia of Immunology, Vol 1. Eds: PJ Delves and IM Roitt.

Sun, X.M., Pang, W., Jia, T., Yan, W.C., He, G., Hao, L.L., Bentué, M. and Suo, X. (2009). Prevalence of *Eimeria* species in broilers with subclinical signs from fifty farms. *Avian diseases*, *53*(2), 301-305.

Sundar, S. B., Harikrishnan, T. J., Latha, B. R., Chandra, G. S., and Kumar, T. M. S. A. (2017). Anticoccidial drug resistance in chicken coccidiosis and promising solutions: A review. *Journal of Entomology and Zoology Studies*, *5*(4), 1526-1529.

Trujillo-Peralta, C., Ashcraft, A., Señas-Cuesta, R., Coles, M., Hernandez-Velasco, X., Selby, C., Forga, A., Tellez-Isaias, G., Vuong, C., Bielke, L. and Barta, J. (2023). Research Note: Isolation, speciation, and anticoccidial sensitivity of spp. recovered from wild turkey feces In The US: Immunology, Health, and Disease. *Poultry Science*, 102819.

USDA Economics, Statistics, and Market Information System. (April 2023). Poultry and Egg Outlook. USDA Economic Research Service. Retrieved from https://usda.library.cornell.edu/concern/publications/m039k491c?locale=en

USDA, National Agricultural Statistics Service. (2023). Poultry - Production and Value 2022 Summary.

Usman, J. G., Gadzama, U. N., Kwaghe, A. V., and Madziga, H. A. (2011). Anticoccidial resistance in poultry: A review. *New York Science Journal*, 4(8), 102-109.

Venkateswara Rao, P., Raman, M., and Gomathinayagam, S. (2015). Sporulation dynamics of poultry *Eimeria* oocysts in Chennai. *Journal of Parasitic Diseases*, *39*, 689-692.

Venugopal, K., Hentzschel, F., Valkiūnas, G., and Marti, M. (2020). Plasmodium asexual growth and sexual development in the haematopoietic niche of the host. *Nature Reviews Microbiology*, *18*(3), 177-189.

Vrba, V., and Pakandl, M. (2014). Coccidia of turkey: From isolation, characterisation and comparison to molecular phylogeny and molecular diagnostics. *International Journal for Parasitology*, *44*(13), 985–1000.

Wang, Z., Suo, X., Xia, X., and Shen, J. (2006). Influence of monensin on cation influx and Na+-K+-ATPase activity of *Eimeria* tenella sporozoites in vitro. *Journal of Parasitology*, *92*(5), 1092-1096.

Williams, R. B. (1995). Epidemiological studies of coccidiosis in the domesticated fowl (Gallus gallus): II. Physical condition and survival of *Eimeria* acervulina oocysts in poultry-house litter. *Applied parasitology*, *36*(2), 90-96.

Williams, R. B. (1999). A compartmentalised model for the estimation of the cost of coccidiosis to the world's chicken production industry. *International journal for parasitology*, *29*(8), 1209-1229.

Williams, R. B. (2002). Anticoccidial vaccines for broiler Chicken: pathways to success. *Avian Pathology*, *31*(4), 317-353.

Williams, R. B. (2006). Tracing the emergence of drug-resistance in coccidia (*Eimeria* spp.) of commercial broiler flocks medicated with decoquinate for the first time in the United Kingdom. *Veterinary Parasitology*, *135*(1), 1-14.

Xiang-hua, L. (2001). Rapid Development of Drug-resistant Strains of tenella to Diclazuril and Halofugenone in Laboratory. Medicine, Biology.

Xiao, L., Sulaiman, I.M., Ryan, U.M., Zhou, L., Atwill, E.R., Tischler, M.L., Zhang, X., Fayer, R. and Lal, A.A. (2002). Host adaptation and host–parasite co-evolution in Cryptosporidium: implications for taxonomy and public health. *International journal for parasitology*, *32*(14), 1773-1785.

Xinyu, P., Jianfei, Z., Wenkang, W., Huixian, W., and Mingquan, X. (2000). Resistance to three chemical anticoccidial drugs of field *Eimeria* tenella isolated from Guangdong Province of China. Zhongguo Shou yi xue bao= *Chinese Journal of Veterinary Science*, *20*(1), 48-50.

Yadav, A., and Gupta, S. K. (2001). Study of resistance against some ionophores in *Eimeria* tenella field isolates. *Veterinary parasitology*, *102*(1-2), 69-75.

Yun, C. H., Lillehoj, H. S., and Lillehoj, E. P. (2000). Intestinal immune responses to coccidiosis. *Developmental & Comparative Immunology*, *24*(2-3), 303-324.

Zaheer, T., Abbas, R. Z., Imran, M., Abbas, A., Butt, A., Aslam, S., and Ahmad, J. (2022). Vaccines against chicken coccidiosis with particular reference to previous decade: progress, challenges, and opportunities. *Parasitology Research*, *121*(10), 2749-2763.

Zhang, D.F., Sun, B.B., Yue, Y.Y., Yu, H.J., Zhang, H.L., Zhou, Q.J. and Du, A.F. (2012). Anticoccidial effect of halofuginone hydrobromide against *Eimeria* tenella with associated histology. *Parasitology research*, *111*, 695-701.

Zidar, P., and Žižek, S. (2012). The impact of coccidiostats monensin and lasalocid on Cd and Pb uptake in the isopod Porcellio scaber. *Applied soil ecology*, *55*, 36-43.

Zigo, F., Ondrašovičová, S., Zigová, M., Takáč, L., and Takáčová, J. (2019). Influence of the flight season on the health status of the carrier pigeons. *Int. J. Avian Wildlife Biol*, *4*(2), 26-30.

Appendix A: Age-Related Impact on Anticoccidial Medication Efficacy in Turkey

Incorporating the context of the age of collection and considering both the random effect of farm and the fixed effect of age on all medications, the regression analysis using a generalized linear model reveals significant effects of age on sensitivity, reduced sensitivity, and resistance. Results revealed that age significantly predicted sensitivity = 3.651, P= 0.02 indicating that sensitivity increased by 3.651% for each additional day of age. Age did not significantly predict reduced sensitivity= -0.004, P= 0.77. For resistance, age effect approached significance = -2.78, P= 0.06, suggesting a trend where resistance decreases as age increases.

Effects of Age of the birds at sampling time on Sensitivity, Reduced Sensitivity, and Resistance

	Sensitivity	*SE*	*P*	*Reduce sensitivity*	*SE*	*P*	*Resistance*	*SE*	*P*
Slope for Age	3.651	0.01359	0.02	-0.004	0.01213	0.77	-02.78	0.0138	0.06

Appendix B: Data on Anticoccidial Efficacy from Current and Previous Studies in Commercial Turkey Production

Study	Medication	Year	n	Sensitive>75%	Partially resistant 51%-74%	Resistant <50%
Abdullah et al., (Chapter 3)	Amprol	2024	24	11.93	33.06	48.65
Abdullah et al., (Chapter 3)	Coyden	2024	24	64.79	21.95	15.04
Abdullah et al., (Chapter 3)	Stenorol	2024	24	39.98	39.34	13.89
Abdullah et al., (Chapter 3)	Avatec	2024	24	05.00	20.55	73.27
Abdullah et al., (Chapter 3)	Coban	2024	24	05.00	45.65	43.74
Abdullah et al., (Chapter 3)	Zoamix	2024	24	02.28	20.55	77.88
Rathinam and Chapman, (2009)	Amprol	2009	33	06.25	0	96.88
Rathinam and Chapman, (2009)	Coyden	2009	33	53.13	31.25	18.75
Rathinam and Chapman, (2009)	Coban	2009	33	12.50	18.75	71.88

Jeffers and Bentley, (1980)	Coban	1980	38	57.89	42.10	0

Appendix C: Medication Sensitivity from Chapter 4

The analysis revealed significant differences (P= 0.02) in the sensitivity of anticoccidial medications against *Eimeria* in turkey. Coyden emerged as the most sensitive treatment significantly outperforming others as shown in Table 4.1. Stenorol also showed good efficacy, whereas Zoamix had the lowest sensitivity.

Table 4.1: Efficacy of Anticoccidial Medication in Turkey

Medication	**LS means**	**SEM**	**P value**
Amprol	33.42	11.75	0.02
Coyden	83.42	11.75	
Stenorol	62.88	12.56	
Avatec	29.21	12.14	
Coban	33.58	11.04	
Zoamix	23.78	11.6	

Discussion

The findings from this study underscore a critical evolution in the control of coccidiosis in commercial turkey populations. The analysis revealed significant differences in the sensitivity of anticoccidial mediations against *Eimeria* in poultry, with a statistically significant difference ($P = 0.02$). Coyden emerged as the most sensitive treatment (LSmean = 83.42, SEM = 11.75), significantly outperforming others. Stenorol also showed good efficacy, whereas Zoamix had the lowest sensitivity (LSmean = 23.78, SEM = 11.6).

Despite the initial success of Amprol the discourse around its long-term efficacy began to shift as early as the 1970s, with emerging research pointing towards the development of resistance. Studies like those conducted by McLoughlin (1970) and Sherkov (1977) revealed that resistance to Amprol not only emerged but could also potentially revert to sensitivity, suggesting a dynamic interaction between the anticoccidal and *Eimeria* that complicates resistance management. This evolving landscape of anticoccidial efficacy was further complicated by the introduction of alternative medications like salinomycin, which initially offered better control of coccidia but soon faced similar challenges of efficacy and resistance (McDougald, 1981; Chapman, 1982).

The transition into the 21st century saw a deeper investigation into the genetics of resistance, inspired by parallels in other fields such as malaria research (Greif et al., 2001). Findings by George and Sabu (2002) indicated that inability of Amprol to contain coccidiosis extended beyond genetic resistance, affecting specific performance parameters, and suggesting a functional decline in anticoccidial efficacy. The comparative analysis between the coccidiocidal effect of Toltrazuril and the coccidiostatic nature of Amprol provided additional insights into the limitations of its mode of action (Lakkundi et al., 2002).

In light of the current results and historical data, it becomes evident that Amprol has shifted from a widely sensitive anticoccidial agent to one facing the specter of increased resistance and encapsulates the broader challenges of managing protozoal infections in commercial turkey. The moderate efficacy observed today, contrasted with the historical context of evolving resistance, underscores the imperative need for continuous innovation in anticoccidial development and strategic application.

The current findings regarding efficacy of Coyden in managing coccidiosis underscores its significant positive impact on sensitivity and minimal contribution to resistance development. Coyden acts on the *Eimeria* lifecycle by targeting the sporozoite and first-generation schizont stages, effectively disrupting early development and controlling coccidiosis progression, as noted by Chapman and Rathinam (2022). The synergy between Coyden and other compounds, such as 4-hydroxyquinolones, further exemplifies the strategic use of combination therapies to enhance efficacy and potentially mitigate the risk of resistance development. The integration of Coyden in combination products, particularly with quinolones, leverages this synergistic potential to achieve comprehensive control of coccidiosis, as documented by Jeffers and Challey (1973).

Further, this study indicates that Stenorol is sensitive against coccidiosis in turkeys. This finding is crucial because both medications have been cornerstone treatments for chickens and turkeys. Though, they have not been applied to poultry production in as high of volume as other anticoccidials due to cost and availability, which has likely contributed to a lack of resistance in commercial turkey operations (Peng, 2000; Huang et al., 2001). Stenorol has remained essential for coccidiosis control due to their unique modes of action, which minimize cross-resistance with other anticoccidials (Peek and Landman, 2010).

The observed lower sensitivity effect of Avatec implies that while it remains sensitive to a degree, its impact is decreasing in the face of evolving strains. Conversely, the tendency towards higher resistance is a clear indicator that continued reliance on Avatec, without strategic management, could lead to a scenario where this its utility is significantly compromised. To counteract this trend, several strategies should be considered. Firstly, the rotation of anticoccidial medications and the implementation of shuttle programs can reduce the selective pressure exerted by continuous use of a single compound like Avatec. Such practices, by introducing variability in the selection environment, can slow down the rate at which resistance develops. Secondly, integrating non-chemical control measures, especially vaccination with anticoccidial sensitive strains, can reduce the risk of resistance development. These alternative strategies not only provide a multifaceted approach to coccidiosis management but also contribute to the sustainability of effective control measures. Lastly, ongoing surveillance and research into the mechanisms of resistance and the efficacy of existing and novel anticoccidial compounds are essential. Understanding the dynamics of anticoccidial sensitivity and resistance at a molecular level can inform the development of next generation anticoccidials designed to overcome current resistance challenges.

Coban has long been utilized in the poultry industry for its efficacy in controlling coccidiosis caused by disrupting the ion balance within parasite cells. However, the concern about emerging resistance is not unfounded and is confirmed in the scientific literature as several studies have illuminated the dynamics of Coban efficacy and the development of resistance. For instance, Chapman et al. (1994) demonstrated that Coban remains sensitive against various strains of *Eimeria* but also noted that continuous use could lead to reduced sensitivity over time. This phenomenon is attributed to the ability of *Eimeria* to gradually adapt to the selective pressure exerted by anticoccidial medications. Ionophores do not fully stop replication of *Eimeria* and are often referred to as “leaky”, meaning that some successful replication continues to occur during treatment cycles. While this has a benefit of allowing birds to develop an immune response to *Eimeria* through leaky exposure, potential that breakthrough *Eimeria* develop resistance to Coban needs to be acknowledged.

Further supporting this, Peek and Landman (2003) observed that while Coban effectively reduces the incidence and severity of coccidiosis, the appearance of Coban-resistant strains on farms using the anticoccidial for extended periods highlights the need for strategic use, including anticoccidial rotation and combination therapy, to manage resistance. This strategic use is aimed at preventing the establishment and spread of resistant strains, thereby prolonging the efficacy of Coban. Moreover, the research by Barnes et al. (2003) on anticoccidial resistance in poultry noted that while resistance to

Coban and other ionophores is less common than to some chemical anticoccidials, it does occur and presents a significant challenge to disease control efforts. The study suggested incorporating non-chemical control measures and monitoring anticoccidial efficacy as part of an integrated approach to coccidiosis management.

Zoamix observed minimal sensitivity in this study spotlighting potential limitations in its long-term effectiveness against *Eimeria* in turkeys. This pattern of diminishing efficacy and increasing resistance underscores a significant concern for turkey producers: the persistent use of Zoamix, without strategic management, can lead to the proliferation of anticoccidial-resistant strains. This issue is particularly acute in turkeys, where the high density of birds and intensive rearing conditions can amplify the spread and impact of resistant parasites. Research involving turkeys has consistently shown that the development of resistance to anticoccidials is not just a possibility but an ongoing challenge that can compromise the control of coccidiosis, impacting flock health and productivity.

These observations underscore the need for a strategic reassessment of how anticoccidial medications are utilized in turkey production. In the wild, where human intervention rarely reaches, the anticoccidial resistance still emerge. The study by Trujillo-Peralta et al. (2023) unveiled that resistance to anticoccidials such as Coban and Zoamix exists in wild turkeys, illustrating that the battle against resistance is not confined to commercial farms. An interesting contrast is observed with Amprol, where sensitivity was reported at 100%. This stark difference in the response to Amprol compared to Coban and Zoamix raises concerns about the origins of resistance and its spread, hinting at environmental factors or transmission between wild and farmed flocks. The increasing anticoccidial resistance among *Eimeria* calls for a judicious approach to anticoccidial use, including the rotation of medications, the integration of non-chemical control measures such as vaccination, and the development of new compounds with novel modes of action.

Conclusion

This study reveals a growing resistance to anticoccidial medications in commercial turkey production, with a marked decline in efficacy, especially for drugs like Zoamix. These trends underscore the urgent need for revised management strategies and the adoption of integrated approaches to coccidiosis control in turkey production.